IN-VITRO AND IN-VIVO BIOAVAILABILITY OF NEVIRAPINE-LOADED HUMAN SERUM ALBUMIN (HSA) NANOPARTICLES

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Submitted by

REG. NO: 26115402

Under the Guidance of

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DEDICATED TO

MY PARENTS

BROTHER

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1.0 ABSTRACT

Bioavailability of nevirapine from the currently available tablet dosage form remains a concern for effective control of HIV infection. The present study attempted to develop nevirapine nanoparticles using human serum albumin (HSA) as polymer in varied concentrations and to evaluate for physico-chemical, in-vitro and in-vivo release characteristics. Three formulations F1, F2, and F3 were developed using drug:polymer ratios 1:1,1:2,and1:4 respectively by desolvation method. The particle size of F1, F2, F3 was 298, 495 and 698nm respectively. The zeta potential of F1, F2 and F3 was -0.27,-0.22and-0.18 mV respectively The PDI of all formulations was less than 0.5 showing homogenous dispersion of nevirapine. The dissolution and diffusion profile of the drug showed that F1 was found to meet the requirement of not less than 85% drug release at 24h with zero order kinetic (r^2 0.99). The Cmax, AUC_{0-24}, and $AUC_{0-\infty}$ were significantly ($p<0.001$) higher than that of neviripine alone. Significant change in Tmax of F1 was observed as compared to nevirapine alone. The results of the study revealed that nevirapine nanoparticle is beneficial in improving the bioavailability of the drug. It can be conclusively stated that nevirapine loaded - HSA nanoparticles may be better than the conventional tablet dosage form for the treatment of HIV infection.

2.0 INTRODUCTION

Viral disease is one of the most prevalent diseases in the modern world. Many viruses eventually kill their host cells, resulting in disease and provoking an assault by the immune response of the host. The viral diseases are commonly occurring worldwide Acquired immunodeficiency syndrome (AIDS), Dengue, Encephalitis, Hepatitis, Yellofever. Among all the viral diseases AIDS is most dangerous and incurable disease. HIV virus comes from the Congo in 1959 and 1960 though genetic studies indicate that it is passed into the human population from chimpanzees around fifty years earlier. A recent study states that a strain of HIV probably moved from Africa to Haiti and then entered united states around 1969. India is one of the largest and most populated countries in the world with over one billion in habitants. Of this number it is estimated that around 2.27 million people are currently living with HIV, which indicates that there are more people with HIV in india than in any other country in the world.[1]

Currently HIV is treated with anti HIV drugs such as lamivudine, stavudine and zidovudine and nevirapine etc. Nevirapine is particularly indicated in the therapy of HIV as non-nucleoside reverse transcriptase inhibitor, and combination of lamivudine, stavudine and nevirapine is recommended for effective control of HIV infection. They are available as tablet dosage forms commercially. However nevirapine being poorly soluble may pose dissolution limited absorption problem resulting in poor bioavailability of the drug and therefore difficulty in controlling HIV infection. Different pharmaceutical approaches are followed to improve the dissolution of NVP. Solid dispersion of NVP with polyvinylpyrolidine (PVP k30) increase the solubility of nevirapine. [2]

Nanoparticles have become one of the most active areas of research in the field of drug delivery due to their ability to deliver drugs to the right place, at appropriate times, and in the right dosage. They have received considerable attention over the past 20 years due to their advantages compared to other drug delivery system. Nanoparticles can be defined as solid micron, colloidal particles ranging in size from 1nm to 1000nm in diameter, generally but not necessarily made of natural or synthetic polymers, in which drugs can be adsorbed, entrapped, encapsulated or covalently attached and are produced by mechanical or chemical means.[3]

A pure anhydrous forn of nevirapine in microspheres was prepared by sublimation and condensation of the drug and was reported 30% more solubule than the pure anhydrous drug and 140%more soluble than the semihydrate form. These microspheres are suitable for formulation into parentral dosage form inhalation therapy and injection.[4] Liposomes of nevirapine were developed using egg phospholipid to cholesterol ratio at 9:1 which shows a prolonged release of NVP upto 1320 min at pH. Nevirapine nanosuspensions for intravenous injection was developed for targeting viral resvoirs in body was assessed the in-vitro protein absorption was carried out using 2-D PAGE. Bare nanosuspensions and surface modified nanosuspension with serum albumin,polysaccharide and PEG were compared regarding there protein absorption patterns. Solid lipid nanoparticles and nanostructured liquid carriers containing nevirapine were reported. These formulations are coated with formulations human serum albumin. An accelerated release of NVP was reported from nanocarriers. When incubated with DODAB-stabilised SLNSs, the viability of human brain micro vascular endothelial cells (HBMECs) reduced. [5]

The in-vitro and in-vivo performance of NPs depends on the type of polymers used in the development of NPs. Biodegradable polymers are most preferred for NPs because these are non toxic easly metabolized and eliminated from the body. Among the biodegradable polymers, Albumin NPs have gained considerable attention owing to their high binding capacity of various drugs and being well tolerated without any serious side effects. [6]

Human serum albumin (HSA) is a promising bio-macromolecule and draws a great attention in both fundamental and applied medication due to its biodegradability, nontoxicity nonimmunogenicity and regulatory function. For example, HSA could stabilize the ingredients in vaccines and modify the surface of medical devices. In addition the antioxidant property of HSA to construct possible innovated structure for therapeutic carriers. In fact HSA were used in frabicating albunex and ABI 007 for clinical purpose. [7]

Nanoparticles are prepared by several methods such as a)Amphiphilic macromolecules crosslinking b)Polymerization based methods c)Polymer precipitation methods d)Ionic gelation method.

Considering the above factors, it is understood that delivery of nevirapine nanoparticles can greatly improve its solubility and improve its bioavailability. Human serum albumin (HSA) is a naturally occurring non immunogenic polymer and is considered advantageous for development of nevirapine nanoparticles. Desolvation method is easy to

follow for the preparation of nanoparticles. Therefore in the present study nevirapine nanoparticles was developed using human serum albumin as a polymer by desolvation method and the nanoparticles was evaluated for physicochemical, in-vitro and in-vivo release characteristics.

3.0 REVIEW OF LITERATURE

Human immunodeficiency virus (HIV) is the causative agent for AIDS. It is a sexually transmitted disease. Infection is aided by Langerhans cells in mucosal epithelial surfaces and by the presence of other sexually transmitted diseases that can produce mucosal ulceration and inflammation. The CD4+ T-lymphocytes have surface receptors to which HIV can attach to promote entry into the cell. The infection extends to lymphoid tissues which contain follicular dendritic cells that can become infected and provide a reservoir for continuing infection of CD4+ T-lymphocytes. When HIV infects a cell, it must use its reverse transcriptase enzyme to transcribe its RNA to host cell proviral DNA. It is this proviral DNA that directs the cell to produce additional HIV virions which are released.[8]

EPIDEMIOLGY OF HIV

The presence of HIV infection among injectors had been reported in 120 of these countries. Prevalence estimates of injecting drug use could be ascertained for 61 countries, containing 77% of the world's total population aged 15–64 years. Extrapolated estimates suggest that 15·9 million (range 11·0–21·2 million) people might inject drugs worldwide; the largest numbers of injectors were found in China, the USA, and Russia, where mid-estimates of HIV prevalence among injectors were 12%, 16%, and 37%, rerespectively.HIV prevalence among injecting drug users was 20–40% in fi ve countries and over 40% in nine. We estimate that, worldwide, about 3·0 million (range 0·8–6·6 million) people who inject drugs might be HIV positive. (**Bradley m mathews et al)** [9]

Worldwide HIV and AIDS Statistics

Table 1

	Estimate (million)	Range (million)
People living with HIV/AIDS in 2008	33.4	31.1-35.8
Adults living with HIV/AIDS in 2008	31.3	29.2-33.7
Women living with HIV/AIDS in 2008	15.7	14.2-17.2
Children living with HIV/AIDS in 2008	2.1	1.2-2.9
People newly infected with HIV in 2008	2.7	2.4-3.0
Children newly infected with HIV in 2008	0.43	0.24-0.61
AIDS deaths in 2008	2.0	1.7-2.4

The genome of HIV contains only three major genes: **env, gag, and pol**. These genes direct the formation of the basic components of HIV. The **env gene** directs production of an envelope precursor protein **gp160** which undergoes proteolytic cleavage to the outer envelope glycoprotein **gp120,** which is responsible for tropism to CD4+ receptors, and transmembrane glycoprotein **gp41**, which catalyzes fusion of HIV to the target cell's membrane. The **gag gene** directs formation of the proteins of the matrix **p17**, the "core" capsid **p24**, and the nucleocapsid **p7**. The **pol gene** directs synthesis of important enzymes including reverse transcriptase **p51** and **p66**, integrase **p32**, and protease **p11 .**[10]

In addition to the CD4 receptor, a coreceptor known as a chemokine is needed for HIV infection. Chemokines are cell surface fusion-mediating molecules. Such coreceptors include CXCR4 and CCR5. Their presence on cells can aid binding of the HIV envelope glycoprotein gp120, promoting infection. Initial binding of HIV to the CD4 receptor is mediated by conformational changes in the gp120 subunit, but such conformational changes are not sufficient of fusion. The chemokine receptors produce a conformational change in the gp41 subunit which allows fusion of HIV. The differences in chemokine coreceptors that are present on a cell also explains how different strains of HIV may infect cells selectively. There are strains of HIV known as T-tropic strains which selectively interact with the CXCR4 chemokine coreceptor to infect lymphocytes. The M-tropic strains of HIV interact with the CCR5 chemokine coreceptor to infect macrophages. Dual tropic HIV stains have been identified.[11]

MECHANISM AND TRANSMISSION OF HIV INFECTION

HIV primarily infects cells with CD4 cell-surface receptor molecules, using them to gain entry (Figure 1). Many cell types share common epitopes with this protein, though CD4 lymphocytes play a crucial role. In macrophages and in some other cells lacking CD4 receptors, such as fibroblasts, an Fc receptor site or complement receptor site may be used instead for entry of HIV. Cells of the mononuclear phagocyte system, principally blood monocytes and tissue macrophages, T lymphocytes, B lymphocytes, natural killer (NK) lymphocytes, dendritic cells (Langerhans cells of epithelia and follicular dendritic cells in lymph nodes), hematopoietic stem cells, endothelial cells, microglial cells in brain, and gastrointestinal epithelial cells are the primary targets of HIV infection .[12]

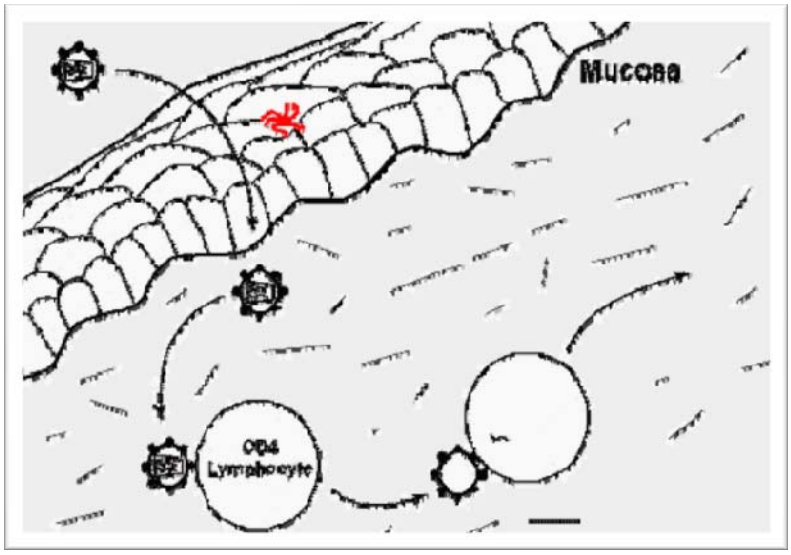

Fig 1 Mechanism of HIV

Human immunodeficiency virus is shown crossing the mucosa of the genital tract to infect CD4+ T-lymphocytes. A Langerhans cell in the epithelium is shown in red in this diagram.[11]

HIV is transmitted through direct contact of a mucous membrane or the bloodstream with a bodily fluid containing HIV, such as blood, semen, vaginal fluid, preseminal fluid, and breast milk. This transmission can involve anal, vaginal or oral sex, blood transfusion, contaminated hypodermic needles, exchange between mother and baby during pregnancy, childbirth, breastfeeding or other exposure to one of the above bodily fluids.

ROLE OF NEVIRAPINE IN HIV INFECTION:

Nevirapine falls in the non-nucleoside reverse transcriptase inhibitor (NNRTI) class of antiretrovirals. Both nucleoside and non-nucleoside RTIs inhibit the same target, the reverse transcriptase enzyme, an essential viral enzyme which transcribes viral RNA into DNA. Unlike nucleoside RTIs, which bind at the enzyme's active site, NNRTIs bind allosterically at a distinct site away from the active site termed the NNRTI pocket.

Nevirapine is not effective against HIV-2, as the pocket of the HIV-2 reverse transcriptase has a different structure, which confers intrinsic resistance to the NNRTI class.

Nevirapine is a prescription medicine approved by the U.S. Food and Drug

Administration (FDA) for the treatment of HIV infection in adults and children. Nevirapine is always used in combination with other anti-HIV medicines. It is a type of anti-HIV medicine called a non-nucleoside reverse transcriptase inhibitor (NNRTI). NNRTIs work by binding to and blocking HIV reverse transcriptase, an HIV enzyme. This prevents HIV from replicating and lowers the amount of HIV in the blood. [13]

Nevirapine does not cure HIV/AIDS. It is not known if nevirapine reduces the risk of passing HIV to other people.

Nevirapine comes in the following forms and strengths:

- 200-mg immediate-release tablets (brand name: Viramune).
- 100-mg extended-release tablets (brand name: Viramune XR).
- 400-mg extended-release tablets (brand name: Viramune XR).
- 50-mg/5 mL oral suspension (brand name: Viramune).

A single dose of nevirapine given to both mother and child reduced the rate of HIV transmission by almost 50% compared with a very short course of zidovudine (AZT) prophylaxis, in a clinical trial in Uganda. A subsequent study in Thailand showed that prophylaxis with single-dose nevirapine in addition to zidovudine is more effective than zidovudine alone. These and other trials have led the World Health Organization to endorse the use of single-dose nevirapine prophylaxis in many developing world settings as a cost-effective way of reducing mother-to-child transmission. However, in the United States the Ugandan study was deemed flawed and as of 2006 the FDA has not approved of such nevirapine prophylaxis.[14] However,supporters of HIVNET 012 experiment argued that the flaws in this experiment were largely due to bureaucratic incompetence, while the findings regarding the safety and efficacy of single-dose nevirapine from this study were scientifically solid and too important to discard. Moreover, it was argued that holding African researchers who operated under resource-poor situations to the same moral and procedural standards to their Western counterparts was unrealistic, and would further marginalize African researchers' role in the science community and impede the progress of African science. Another clinical trial, Using Nevirapine to Prevent Mother-to-Child HIV Transmission During Breastfeeding is scheduled for completion in March 2011.[15]

A major concern with this approach is that NNRTI resistance mutations are commonly observed in both mothers and infants after single-dose nevirapine , and may compromise the response to future NNRTI-containing regimens. A short course of maternal zidovudine/lamivudine is recommended by the U.S. Public Health Service Task Force to reduce this risk.

APPROACHES TO IMPROVE BIOAVAILABILITY OF NEVIRAPINE:

The present work was to improve the solubility of Nevirapine by solid dispersion techniques using Polyvinylpyrrolidone K 30 (PVP K 30) as carrier. The Solid dispersion was prepared by physical mixing, solvent evaporation and kneading method. The interaction of the Nevirapine with PVP K 30 was evaluated by the Fourier transform infrared (FTIR) spectroscopy; Differential scanning Calorimetry (DSC), X-ray diffraction patterns (XRD). The results from the FTIR and XRD analyses showed that Solid dispersion might exist in the amorphous form. A DSC result showed that the sharp melting point was completely disappeared suggesting that the Nevirapine molecularly dispersed in an amorphous form. Saturation solubility and dissolution studies indicate that dissolution rate was remarkably increased in Solid dispersion as compared to the physical mixture and drug alone. In conclusion PVP K 30 can be a well utilized to increase the solubility of poorly water soluble drugs**. (Ahire B. R. et al 2010)** [4]

This invention entails the preparation of very pure nano-and microspheres , measuring approximately 0,5-4 μm of a novel form of nevirapine. The microspheres are prepared without the use of any excipients or other carrier substances. The method involves the sublimation and condensation of the nevirapine raw material without any carrier or excipients,rendering a pure anhydrous form of nevirapine. This new form was found at least 30% more soluble than the anhydrous form and 140% more soluble than the hemihydrate form. The small size of the new form's particles (0,5-4 μm) majes them suitable for suitable for formulation into parentral dosage forms, making inhalation therapy and injection possible. (**Rudi Van Der Walt et al)** [16]

In the present study liposomes of uniform diameters were prepared using thin film hydration and extrusion technique and a hydrophobic non-nucleoside reverse trascriptase inhibitor, nevirapine was successfully encapsulated in the liposomes. The best encapsulation was observed at an egg phospholipid to cholesterol ratio og 9:1 which also showed a prolonged rerelease of nevirapine up to 1320 minutes at physiological ph. Presence of

proteins in the medium and external stimuli like low frequency ultrasound was found to enhaance the rate of drug release. The use of ultrasound leading to higher magnitude of drug release thus points to a potentially novel approach towards anti-retroviral therapy. Presence of cholesterol in the liposomes offers stability against fluidizing actio of proteins without preventing the disruption of the liposomal architecture by ultrasound. **(Lakshmi N Ramana et al)**[5]

NANOTECHNOLOGY

Nanotechnology, the term derived from the Greek word Nano, meaning dwarf, applies the principles of engineering, electronics, physical and material science, and manufacturing at a molecular or submicron level. The materials at nanoscale could be a device or a system or these could be supramolecular structures, complexes or composites.

An early promoter of nanotechnology, Albert Franks, defined it as 'that area of science and technology where dimensions and tolerances are in the range of 0.1nm to 100nm'. Nano technology is expected to make significant advances in the mainstream biomedical applications, including in the areas of gene therapy, drug delivery, imaging, and novel drug discovery techniques. [17]

Nanotechnology is hailed as a new generation of technology with the potential to revolutionise many facets of the world we live in. This includes virtually all aspects of daily life, including health and health care, the manufacturing and use of materials and equipment, the environment and protection thereof. It is said to be able to massively increase manufacturing production at significantly reduced costs. Products of nanotechnology will be smaller, cheaper, lighter yet more functional and require less energy and fewer raw materials to manufacture. However, the 'revolution' will not happen overnight and very large investments in research and development will be required in the process.

Nanotechnology can be defined as having the following features

- It involves research and technology development at the 1 nm–100 nm range
- It creates and uses structures that have novel properties because of their small Size
- It builds on the ability to control or manipulate at the atomic and molecular scale

At the nano-scale the interactions and physics between atoms display 'exotic' properties that are absent at larger scale because at this level atoms leave the realm of classical physical properties behind and enter the realm of quantum mechanics.

Nanotechnology includes a bewildering array of activities including: molecular manufacturing, supramolecular and self assembly/organization; biomimicry; nanoparticles (e.g. Bucky balls and carbon nano tubes), nanospheres, nano cups and nanorods; nanobots (nanorobots); colloids, micelles, vesicles and nano-emulsions; clathrate complexes and intercalation compounds.

The National Science Foundation in the USA predicts that the global marketplace for goods and services using nanotechnologies will grow to $1 trillion by 2015, and there are already over 500products being sold that claim they are made with nanoscale or engineered Nanomaterials. These include products like self-cleaning windows, automobile paint, sunscreens, and tennis rackets. In the future, a marriage of nano- and biotechnology will likely create a whole new generation of drugs, biomedical devices, and other solutions to some of our most challenging medical problems[18].

Nanotechnology in drug delivery:

The development of delivery systems for small molecules, proteins and DNAhas been impacted to an enormous degree over the past decade by nanotechnology, and has led to the development of entirely new and somewhat unpredicted fields. For the pharmaceutical industry, novel drug delivery technologies represent a strategic tool for expanding drug markets. The technology can address issues associated with current pharmaceuticals such as extending product life (line extension), or can add to their performance and acceptability, either by increasing efficacy or improving safety and patient compliance[19]. This technology is permitting the delivery of drugs that are highly water- insoluble or unstable in the biological environment. Advantages of Nano sizing of drugs has the potential to: Increase surface area, enhance solubility, increase rate of dissolution, increase oral bioavailability, more rapid onset of therapeutic action, decrease the dose needed, decrease fed/fasted variability and decrease patient to patient variability.

In recent trend, Nano drug delivery may occur through gold nanospheres and rods, nanowires, nanotriangles, nanostars, nanocubes, and nanorice. The size of these nano configurations varies from 1 to 100 nm. Nanoplatforms include organic nanostructures, polymeric nanoparticles, lipid systems-liposomes, self assemblies-micelles, dendrimers, and

carbon nanostructure-nanotubes. Inorganic nanostructures include metal nanoparticles and nanoshells, silicon nanostructure, nanocrystals, and quantum dots. Hybrid nanostructures, combining two to three of those previous listed can also be produced. Studies were described in which polymeric nanoparticles were used for tumor-targeted deliver. Gelatin-based engineered nanoparticles have been used for gene delivery and multifunctional nanoemulsions for oral and intravenous delivery. Gadolinium-loaded nanoemulsion has been used in animals for brain imaging, and this technology could easily be used for imaging within the eye to observe the results of various drug delivery modalities.

The benefits of Nanotechnology are:

- The lifespan of the blockbuster drugs can be resurrected by reformulating the
- drugs through novel drug delivery system.
- The effective patent protection can be enhanced.
- Drug delivery formulation involves low cost research compared to that for the
- discovery of new molecules.
- Minimizing use of expensive drugs would reduce the cost of the product.

NANOPARTICLES

Nanoparticles are solid colloidal particles with diameters ranging from 1-1000 nm. They consist of macromolecular materials and can be used therapeutically as adjuvant in vaccines or drug carriers, in which the active ingredient is dissolved, entrapped, encapsulated, adsorbed or chemically attached. Polymers used to form nanoparticles can be both synthetic and natural polymers. There are two types of nanoparticles depending on the preparation process nanospheres and nanocapsules. Nanospheres have a monolithic-type structure (matrix) in which drugs are dispersed or adsorbed on to their surfaces. Nanocapsules exhibit a membrane-wall structure and drugs are entrapped in the core or adsorbed on to their exterior .The term “nanoparticles” is adopted because it is often very difficult to unambiguously establish whether these particles are of a matrix or a membrane type.[20] In recent years, biodegradable polymeric nanoparticles, particularly those coated with hydrophilic polymer such as poly (ethylene glycol) (PEG) known as long-circulating particles, have been used as potential drug delivery devices because of their ability to circulate for a prolonged period

time, target a particular organ, as carriers of DNA in gene therapy, and their ability to deliver proteins, peptides and genes.[21]

The major goals in designing nanoparticles as a delivery system are to control particle size, surface properties and release of pharmacologically active agents in order to achieve the site-specific action of the drug at the therapeutically optimal rate and dose regimen. Though liposomes have been used as potential carriers with unique advantages including protecting drugs from degradation, targeting to site of action and reduction toxicity or side effects, their applications are limited due to inherent problems such as low encapsulation efficiency, rapid leakage of water-soluble drug in the presence of blood components and poor storage stability. On the other hand, polymeric nanoparticles offer some specific advantages over liposomes. For instance, they help to increase the stability of drugs/proteins and possess useful controlled release properties[22]

The Advantages of using nanoparticles as a drug delivery system include the following

- Particle size and surface characteristics of nanoparticles can be easily manipulated to achieve both passive and active drug targeting after parenteral administration.
- They control and sustain release of the drug during the transportation and at the site of localization, altering organ distribution of the drug and subsequent clearance of the drug so as to achieve increase in drug therapeutic efficacy and reduction in side effects.
- Controlled release and particle degradation characteristics can be readily modulated by the choice of matrix constituents. Drug loading is relatively high and drugs can be incorporated into the systems without any chemical reaction; this is an important factor for preserving the drug activity.
- Site-specific targeting can be achieved by attaching targeting ligands to surface of particles or use of magnetic guidance.
- The system can be used for various routes of administration including oral, nasal, parenteral, intra-ocular etc.

In spite of these advantages, nanoparticles do have limitations. For example, their small size and large surface area can lead to particle particle aggregation, making physical handling of nanoparticles difficult in liquid and dry forms. In addition, small particles size and large surface area readily result in limited drug loading and burst release. These practical

problems have to be overcome before nanoparticles can be used clinically or made commercially available.

Related works on nanoparticles with anti hiv drugs:

In the present study, an attempt was made to develop nanoparticulate delivery system for highly water soluble drug lamivudine. Chitosan nanoparticles of drug lamivudine were prepared by ionic gelation technique. The method was able to produce discrete, free flowing and uniform sized particles. All the formulations showed high process yield and drug loading capacity. Among the different batches, Formulation F1 (drug polymer ratio 1:1) was selected as the ideal formulation, after considering their better drug loading capacity, and *in vitro* drug release. Based on the observations, it can be concluded that the formulated nanoparticulate delivery system of highly water soluble drug lamivudine using widely accepted and physiologically safe polymer was capable of exhibiting sustained release properties for a period of 24 h. They are thus may be reduce frequency of dosing, thereby minimizing the occurrence of side effects, improve bioavailability and increase the effectiveness of the drug**. (Dhanush et al)** [23]

Albumin nanoparticles of anti viral drug azidothymidine were prepared and evaluated for brain specific delivery after intravenous administration. Long circulatory polyethyleneglycolated (PEGylated) albumin nanoparticles of azidothymidine were prepared by ultra-emulsification method using chemical cross linking by glutaraldehyde. Surface of PEGylated nanoparticles was modified by anchoring teansferrin as a ligand for brain targeting. Fluorescence studies revealed the enhanced uptake of transferring-anchored nanoparticles in brain tissue when compared with unmodified nanoparticles. A significant enhancement of brain localization of azidothymidine was observed for transferring anchored PEGylated albumin nanoparticles **(vivek m et al.,).**[24]

In the study of nevirapine nanosuspensions were prepared by high-presssure homogenization characterized. A crystalline NS of nevirapine for intravenous injection was developed assessed regarding its targeting potential to viral reservoirs in body .To determine the intereactions of the nanocrystals with proteins,in vitro protein absorption studies in plasma were carried out using 2-D PAGE. The in vitro protein rejecting and accepting

proteins were studied as a function of stabilizer of the nanocrystals Bare NS and surface modified NS (eg serum albumin, polysaccharide and PEG) were compared regarding their protein absorption patterns**.** **(Ranjitha shegokar et al 2011)** [25]

Solid lipid nanoparticles(SLNs) and nanostructured lipid carriers(NLCs) coated with human serum albumin(HSA) were fabricated for formulating nevirapine(NVP). Here NLCs contained low melting point oleic acid(OA) in the internal liquid phase.The results revealed that the two nanoparticles were uniformly distributed with th average diameter ranging from 145 to 180nm. The surface HSA neutralized the positive charge of dimethyldioctadecyl ammonium bromide (DODAB) on SLNs and NLCs and reduced their zeta potential. In a fixed ratio of solid lipids, SLNs entrapped more NVP than NLC. The incorporation of OA also reduced the thermal resistance of NLCs and accelerated the release of NVP from the nanocarriers. When with DODAB-stabilized SLNs, the viability of human brain microvascular endothelial cells reduced. However the surface HSA increased the viability of HBMECs about 10% when the cancentration of SLNs was higher than 0.8mg/ml. HSA grafted SLNs and NLCs can be effective formulations in the delivery of NVP for viral therapy. **(Yung-chih kuo et al 2010)** [26]

Table 2

POLYMERS USED FOR THE PREPARATION OF NANOPARTICLES[27]

Polymer use	Technique	Candidate drug
Hydrophilic		
Albumin, gelatin	Heat denaturation and cross-linking in w/o emulsion	Hydrophilic
	Desolvation and cross-linking in aqueous medium	Hydrophobic and protein affinity
Alginate, chitosan	Cross-lining in aqueous medium polymer precipitation in an organic solvent	Hydrophilic
Dextarn		Hydrophilic
Hydrophobic	Emulsion polymerization	
Poly(alkylcyanoacrylates)	Interfacial o/w polymerization	Hydrophilic
		Hydrophobic
	Solvent extraction-evaporation	
Polyesters		Hydrophilic& Hydrophobic
Poly (lactic acid), poly (lactide-co-glycolide), poly (e-cprolactone)	Solvent displacement	Soluble in polar solvent
	Salting out	Soluble in polar solvent

These methodologies are conveniently classified as follow

1) Amphiphilic macromolecules cross-linking

a) Heat cross-linking

b) Chemical cross-linking

2) Polymerization based methods

a) Polymerization based methods

b) Emulsion (micellar) polymerization

c) Dispersion polymerization

d) Interfacial condensation polymerization

e) Interfacial complexation

3) Polymer precipitation methods

a) Solvent extraction/evaporation

b) Solvent displacement (nanoprecipitation)

c) Salting out

4) Ionic gelation method

Nanoparticles can be prepared from a variety of materials such as proteins, polysaccharides and synthetic polymers. The selection of matrix materials is dependent on many factors including[28]

a) Size of nanoparticles required.

b) Inherent properties of the drug e.g., aqueous solubility and stability.

c) Surface characteristics such as charge and permeability.

d) Degree of biodegradability, biocompatibility and toxicity.

e) Drug release profile desired and

f) Antigencity of the final product.

Nanoparticles have been prepared most frequency by three methods.

1) Dispersion of preformed polymers.

2) Polymerization of monomers; and

3) Coacervation.

How ever other methods such as superficial fluid technology and particle replication in non-wetting templates have also been described in the literature for production of nanoparticles. The latter was claimed to have absolute control of particle size, shape and composition, which could set an example for the future mass production of nanoparticles in industry. Dispersion of preformed polymers is a common technique used to prepare

biodegradable nanoparticles from poly (lactic acid) (PLA); poly (D, L-glycoside), PLG; poly (D, L-lactide-co-glycolide) (PCA) 21. This technique can be used in various ways as described below.

Solvent evaporation method:

In this method, the polymer is dissolved in an organic solvent such as dichloromethane, chloroform or ethyl acetate, which is also used as the solvent for dissolving the hydrophobic drug. The mixture of polymer and drug solution is then emulsified in an aqueous solution containing a surfactant or emulsifying agent to form oil in water (o/w) emulsion. After the formation of stable emulsion, the organic solvent is evaporated either by reducing the pressure or by continuous stirring. Particle size was found to be reducing the pressure or by continuous stirring. Particle size was found to influence by the type and concentrations of stabilizer, homogenizer speed and polymer concentration. In order to produce small particle size, often a high-speed homogenization or ultrasonication may be employed.[29]

Spontaneous emulsification or solvent diffusion method:

This is a modified version of solvent evaporation method19. In this method, the water miscible solvent along with a small amount of the water immiscible organic solvent is used as an oil phase. Due to the spontaneous diffusion of solvents an interfacial turbulence is created between the two phases leading to the formation of small particles. As the concentration of water miscible solvent increases, a decrease in the size of particle can be achieved. Both solvent evaporation and solvent diffusion methods can be used for hydrophobic or hydrophilic drugs. In the case of hydrophilic drug, a multiple w/o/w emulsion needs to be aqueous phase.[30]

Polymerization method

In this method, monomers are polymerized to form nanoparticles in an aqueous solution. Drug is incorporated either by being dissolved in the polymerization medium or by adsorption onto the nanoparticles after polymerization completed. The nanoparticle suspension is then purified to remove various stabilizers and surfactants employed for polymerization by ultracentrifugation and re-suspending the particles in an isotonic surfactant-free medium. This technique has been reported for making polybutylcyanoacrylate or poly (alkylcyanoacrylate) nanoparticles. Nanocapsules formation and their particle size depend on the concentration of the surfactants and stabilizers used.[31,32]

Cocervation or ionic gelation method

Much research has been focused on the preparation of nanoparticles using biodegradable hydrophilic polymers such as Chitosan, gelatin and sodium alginate. Calvo and co-workers developed a method for preparing hydrophilic Chitosan nanoparticles by ionic gelation. The method involves a mixture of two aqueous phases, of which one is the polymer chitosan, a di-block co-polymer ethylene oxide or propylene oxide (PEOPPO) and the other is a polyanions sodium tripolyphosphate. In this method, positively charged amino group of chitosan interacts with negative charged tripolyphosphate to form coacervates with a size in the range of nanometer. Coacervates are formed as a result of electrostatic interaction between two aqueous phases, whereas, ionic gelation involves the material undergoing transition from liquid to gel due to ionic interaction conditions at room temperature.[31]

Production of nanoparticles using superficial fluid technology[33]

Conventional methods such a solvent extraction-evaporation, solvent diffusion and organic phase separation methods require the use of organic solvents which are hazardous to the environment as well as to physiological systems. Therefore, the superficial fluid technology has been investigated as an alternative to prepare biodegradable micro and nanoparticles because supercritical fluids are environmentally safe.

A superficial fluid can be generally defined as a solvent at a temperature above its critical temperature, at which the fluid remains a single phase regardless of pressure. Supercritical CO_2 (SC CO_2) is the most widely used supercritical fluid because of its mild critical conditions (Tc = 31.1^0C, Pc = 73.8 bars), non-toxicity, non-inflammability, and low price. The most common techniques involving supercritical fluids are supercritical anti-solvent (SAS) and rapid expansion of critical solution. The process of SAS employs a liquid solvent, e.g. methanol, which is completely miscible with the supercritical fluid to dissolve the solute to be micronized; at the process conditions, because the solute is insoluble in the supercritical fluid, the extract of the liquid solvent by supercritical fluid leads to the instantaneous precipitation of the solute, resulting the formation of nanoparticles. Thote and gupta (2005) reported the use of a modified SAS method for formation of hydrophilic drug dexamethasone phosphate drug nanoparticles for micro encapsulation purposes.

Polyelectrolyte complex (PEC) [34]

Polyelectrolyte complex or self assemble polyelectrolyte is a term to describe complexes formed by self-assembly of the cationic charged polymer and plasmid DNA.

Mechanism of PEC formation involves charge neutralization between cationic polymer and DNA leading to a fall in hydrophilicity as the polyelectrolyte component self assembly. Several cationic polymers (i.e. gelatin, polyethylenimine) also possess this property. Generally, this technique offers simple and mild preparation method without harsh conditions involved. The nanoparticles spontaneously formed after addition of DNA solution into Chitosan dissolved in acetic acid solution, under mechanical stirring at or under room temperature (Erbacher *et al*., 1998). The complexes size can be varied from 50 nm to 700 nm.

Microemulsion method[35]

Chitosan NP prepared by microemulsion technique was first developed by Maitra *et* 1999).This technique is based on formation of chitosan NP in the aqueous core of reverse micellar droplets and subsequently cross-linked through glutaraldehyde. In this method, a surfactant was dissolved in N-hexane. Then, chitosan in acetic solution and glutaraldehyde were added to surfactant/hexane mixture under continuous stirring at room temperature. Nanoparticles were formed in the presence of surfactant. The system was stirred overnight to complete the cross-linking process, which the free amine groups of chitosan conjugate with glutaraldehyde. The organic solvent is then removed by evaporation under low pressure. The yields obtained were the cross-linked chitosan NP and excess surfactant. The excess surfactant was then removed by precipitate with CaCl2 and then the precipitant was removed by centrifugation. The final nanoparticles suspension was dialyzed before Lyophilization. This technique offers a narrow size distribution of less than 100 nm and the particle size can be controlled by varying the amount of glutaraldehyde that alters the degree of cross-linking. Nevertheless, some disadvantages exist such as the use of organic solvent, time-consuming preparation process, and complexity in the washing step.

Freeze drying of nanoparticles[36]

Protective excipients, such as carbohydrates, are widely used in freeze-drying to ensure redispersibility and to avoid aggregation or size changes of nanoparticles[50]. Glucose and lactose were evaluated as cryo- and lyo protectants for the L- PLA nanoparticles because these nanoparticles could not survive during the drying process without protectants. Even the smallest tested amount of glucose (weight ratio glucose: nanoparticles 1:4) was found to protect the nanoparticles, although the appearance of the dried material was translucent and sticky, and its redispersibility was poor. When lactose was used as a protectant, it enhanced the appearance of the cake (the dried material) as a white powder, eligible for a freeze-dried

formulation. Redispersion of the nanoparticle was possible, but as a form of visible aggregates. Further freeze-thawing experiments revealed that already the freezing step (with lactose) destroyed the particles. Next, the two carbohydrates were used together to combine the cryoprotective functionality of glucose and the lyoprotective functionality of lactose. The best result, prolonged Tyndall effect (opalescence in the dispersion) after redispersion of the dried formulation and good quality nanoparticles were obtained, when the amount of lactose was double the amount of glucose. The weight ratios of glucose and lactose to the nanoparticles were 1:2 and 1:1, respectively. Additionally, when an extra stabilizer, Tween 80, was used during the nanoparticle preparation or during the redispersion, the freeze-dried cake could be redispersed more easily with increased stability (prolonged Tyndall effect).

The good cryoprotective results with glucose probably arise from its ability to bind water molecules to the amorphous phase which it forms during the freezing step. Part of the water in the frozen glucose remained non-frozen (even 32% w/w). That water acted as a plasticizer and as a spacing matrix reducing the pressure of ice crystals against the nanoparticles and preventing harmful aggregation caused by freeze concentration, respectively. At the same time, insufficient cryoprotective function of lactose derived most likely from its lower water binding activity. However, as a combination with glucose, lactose reduced the amount of water to a level where the interaction of glucose with water was reduced and, thus, the formation of ice crystal was slightly promoted. This enabled sufficient evaporation of water during the drying and formation of a proper cake. Tween 80 improved the freeze-drying result as it acted as a steric stabilizer and increased the hydrophilicity of the nanoparticles. A hydrophilic surface enhances the redispersion properties of the freeze-dried nanoparticles [37,38].

This technique involves the freezing of the nanoparticle suspension and subsequent sublimation of its water content under reduced pressure to get a free flowing powdered material following advantages are cited for the freeze drying of nanopartilces[29]:

- Prevention from degradation and/or solubilization of the polymer.
- Prevention from drug leakage, drug desorption and/or drug degradation.
- Easy to handle and store and helps in long-term prevention/conservation of nanoparticles.

Readily dispersible in water without modification in their physicochemical properties.

Criteria for ideal polymeric carriers for nanoparticles & nanoparticle delivery systems[39]

Polymeric carriers

- Easy to synthesize and characterize
- Inexpensive
- Biocompatible
- Biodegradable
- Non-immunogenic
- Non-toxic
- Water soluble

Nanoparticle delivery systems

- Simple and inexpensive to manufacture and scale-up
- No heat, high shear forces or organic solvents involved in their preparation process.
- Reproducible and stable
- Applicable to a broad category of drugs; small molecules, proteins and polynucleotides
- Ability to lyophilize
- Stable after administration
- Non-toxic

Table 3

CHARACTERISTICS OF NANOPARTICLES ON DRUG DELIVERY

DIFFERENT PARAMETERS AND CHARACTERIZATON METHODS OF NANOPATICLES[29]

Parameter	Characterization method
Particle size and distribution	Photo correlation spectroscopy (PCS) Laser defractometry Transmission electron microscopy(TEM) Scanning electron microscopy(SEM) Mercury porositometry
Charge determination	Laser Doppler anemometry Zeta potentiometer
Surface hydrophobicity	Water Doppler Anemometry Rose Bengal (dye) binding Hydrophobic interaction chromatography X-ray photoelectron spectroscopy
Chemical analysis of surface	Static secondary ion mass spectroscopy
Carrier-drug interaction	Differential scanning calorimetry (DSC)
Nanoparticles dispersion stability	Critical flocculation temperature (CFT)
Release profile	*In vitro* release characteristics under physiologic and sink conditions
Drug stability	Bioassay of drug extracted from nanoparticles chemical analysis of drug

Particle Size

Particle size and size distribution are the most important characteristics of nanoparticle systems. They determine the *in vivo* distribution, biological fate and targeting ability of nanoparticle systems. In addition they can also influence the drug loading, drug release and stability of nanoparticles.

Many studies have demonstrated that nanoparticles of sub-micron size have a number of advantages over microparticles as a drug delivery system[44]. Generally nanoparticles have relatively higher intracellular uptake compared to microparticles and available to wider range of biological targets due to their small size and relative mobility.100nm nanoparticles had a 2.5 fold greater uptake than 1μm microparticles and 6 fold greater uptakes than 10μm microparticles in a CACO-2 cell line (Desai et al 1996). In a subsequent study, the nanoparticles penetrated through out the sub mucosal layers in a rat in situ intestinal loop model, while microparticles were predominantly localized in the epithelial lining. It was also reported that nanoparticles can cross the blood-brain barrier following the opening of tight junctions by hyper osmotic mannitol, which may provide sustained delivery of therapeutic agents for difficult-to-treat diseases like brain tumors. Tween 80 coated nanoparticles have been showed to cross the blood-brain barrier. In some cell lines, only submicron nanoparticles can be taken up effectively but not the larger size microparticles. The recent literature shows ophthalmic nanosuspension that proves to be boon for drugs that exhibit poor soluble in lachrymal fluid.

Drug release is affected by particle size. Smaller particles have larger surface area; therefore, most of the drug associated would be at or near the particle surface, leading to fast drug release. The particle surface, leading to fast drug release. Whereas, larger particles have large cores which allow more drug to be encapsulated and slowly diffuse out[40].

Currently, the fastest and most routine method of determining particle size is by photon-correlation spectroscopy or dynamic light scattering. Photon-correlation spectroscopy requires the viscosity of the medium to be known and determines the diameter of the particle by Brownian motion and light scattering properties[41]. The results obtained by photon-correlation spectroscopy are usually verified by scanning or transmission electron microscopy (SEM or TEM).

Surface Properties of Nanoparticles

When nanoparticles were administered intravenously, they are easily recognized by the body immune systems, and are then cleared by phagocytes from the circulation. A part from the size of nanoparticles, their surface hydrophobicity determines the amount of adsorbed blood components, mainly proteins. This is turn influences the *in vivo* fate of nanoparticles. Binding of these opsonins onto the surface of nanoparticles called opsonization acts as a bridge between nanoparticles and phagocytes. The association of a drug to conventional carriers leads to modification of the drug biodistribution profile, as it is mainly delivered to the mononuclear phagocytes system such as liver, spleen, lungs and bone marrow. Indeed, once in the blood stream, surface non-modified nanoparticles are rapidly opsonized and massively cleared by the macrophages of mononuclear phagocytes system rich organs. Generally, it is IgG, compliment C_3 components that are used for recognition of foreign substances, especially foreign macromolecules.

Hence, to increase the likelihood of the success in drug targeting by nanoparticles, it is necessary to minimize the opsonized and to prolong the circulation of nanoparticles *in vivo*. This can be achieved by surface coating of nanoparticles with biodegradable copolymers with hydrophilic segments such as polyethylene glycol (PEG) Polyethylene oxide, polyoxamer, poloxamine and polysorbate 80 (Tween 80).

The zeta potential of a nanoparticle is commonly used to characterize the surface charge property of nanoparticles[42]. It reflects the electrical potential of particles and is influenced by the composition of the particle and the medium in which it is dispersed. Nanoparticles with a zeta potential above (+/-) 30 mV have been shown to be stable in suspension, as the surface charge prevents aggregation of the particles. The zeta potential can also be used to determine whether a charged active material is encapsulated within the centre of the *nanocapsule* or adsorbed onto the surface.

Drug-polymer interactions[43]

Drug loading can be performed during the preparation of nanoparticles or by adsorbing/absorbing in preformed particles. Within the particle-forming polymer, drug can be present as a solid solution (individual drug molecules) or as a solid dispersion (amorphous/crystalline drug). It can be adsorbed on the particle surface or bound chemically within the nanoparticles .The preparation process can also modify the crystal structure of the drug. The polymer is usually amorphous or semi-crystalline. Differential scanning calorimetry (DSC), (powder) x-ray diffractometry (XRPD) and FTIR are commonly used techniques to reveal the physicochemical state and possible interactions of the drug and the polymer in pharmaceutical micro- and nanoparticles. Polymer MW is determined e.g. by size exclusion chromatography (SEC) the term gel permeation chromatography (GPC) is interchangeably used.

DSC detects phase transitions such as glass transition, (exothermic) crystallization and (endothermic) melting: the nanoparticle sample is heated and changes in heat flow, compared to reference, are registered .Crystallinity/amorphicity properties are obtained from XRPD analysis when diffraction pattern of the x-ray from the sample is determined as a function of scattering angle.. In FTIR, a vibrational spectrum, characteristics for a given crystal structure, is obtained.

Absence of the drug melting peak and diffraction peaks of the crystal structure of the drug in DSC thermo gram and XRPD pattern, respectively, are usually signs of amorphous or molecularly dispersed drug within the polymer .It can also indicate that the amount of drug is lower than the detection limit of the instrument .Drug polymer interactions (e.g. plasticizing effect of drug on polymer) or polymorph change of the drug can be detected as peak shifts in DSC thermogram, band shifts in FTIR spectra or as new reflections in XRPD pattern. Correspondingly, smoothened XRPD pattern, increased cold crystallization exotherms (DSC) or some band shifts to higher wave numbers (FTIR) indicate increased amorphicity of the polymer.

Drug loading[44]

Ideally, a successful nanoparticulate system should have a high drug-loading capacity thereby reduce the quantity of matrix materials for administration. Drug loading can be done by two methods:

- Incorporating at the time of nanoparticles production (incorporation method)
- Absorbing the drug after formation of nanoparticles by incubating the carrier with a concentrated drug solution (adsorption /absorption technique). Drug loading and entrapment efficiency very much depend on the solid-state drug solubility in matrix material or polymer (solid dissolution or dispersion), which is related to the polymer composition, the molecular weight, the drug polymer interaction and the presence of end functional groups (ester or carboxyl). The PEG moiety has no or little effect on drug loading. The macromolecule or protein shows greatest loading efficiency when it is loaded at or near its isoelectric point when it has minimum solubility and maximum adsorption For small molecules, studies show the use of ionic interaction between the drug and matrix materials can be a very effective way to increase the drug loading.[45,46]

***In-vitro* Drug release**[44]

To develop a successful nanoparticulate system, both drug release and polymer biodegradation are important consideration factors. In general, drug release rate depends on: (1) solubility of drug; (2) desorption of the surface bound/adsorbed drug; (3) drug diffusion through the nanoparticle matrix; (4) nanoparticle matrix erosion/degradation; and (5) combination of erosion/diffusion process. Thus solubility, diffusion and biodegradation of the matrix materials govern the release process.

In the case of nanospheres, where the drug is uniformly distributed, the release occurs by diffusion or erosion of the matrix under sink conditions. If the diffusion of the drug is faster than matrix erosion, the mechanism of release is largely controlled by a diffusion process. The rapid initial release or 'burst' is mainly attributed to weakly bound or adsorbed drug to the large surface of nanoparticles[47]. It is evident that the method of incorporation has an effect on release profile. If the drug is loaded by incorporation method, the system has a relatively small burst effect and better sustained release characteristics. If the nanoparticle is coated by polymer, the release is then controlled by diffusion of the drug from the core across the polymeric membrane. The membrane coating acts as a barrier to release, therefore, the

solubility and diffusivity of drug in polymer membrane becomes determining factor in drug release. Furthermore release rate can also be affected by ionic interaction between the drug and addition of auxillary ingredients. When the drug is involved in interaction with auxillary ingredients to form a less water soluble complex, then the drug release can be very slow with almost no burst release effect; whereas if the addition of auxillary ingredients e.g., addition of ethylene oxide-propylene oxide block copolymer (PEO-PPO) to chitosan, reduces the interaction of the model drug bovine serum albumin (BSA) with the matrix material (chitosan) due to competitive electrostatic interaction of PEO-PPO with chitosan, then an increase in drug release could be observed[48].

Various methods which can be used to study the *in vitro* release of the drug are: (1) side-by-side diffusion cells with artificial or biological membranes; (2) dialysis bag diffusion technique; (3) reverse dialysis bag technique; (4) agitation followed by ultracentrifugation/centrifugation; (5) Ultra-filtration or centrifugal ultra-filtration techniques. Usually the release study is carried out by controlled agitation followed by centrifugation. Due to the time-consuming nature and technical difficulties encountered in the separation of nanoparticles from release media, the dialysis technique is generally preferred.

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4.0 PROFILES

4.1 DRUG PROFILE

NEVIRAPINE [51,52] :

Category: Anti retroviral agent

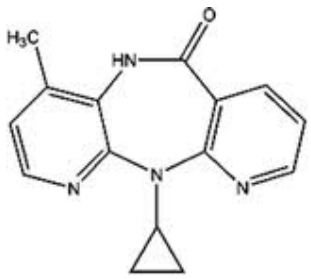
Empirical formula: $C_{15}H_{14}N_4O$

Molecular weight: 266.3

Physical properties: White to all most white crystalline powder

Solubility: Practically insoluble in water. Sparingly soluble or slightly soluble in dichloromethane. Slightly soluble in methyl alcohol.

Chemical structure:



11-cyclopropyl-4-methyl-5,11-dihydro-6H-dipyrido[3,2-b:2,3-e][1,4]diazepin-6-one.

Mechanism of action:

Nevirapine diffuses into the cell and binds to reverse transcriptase adjacent to the catalytic site and it leads to conformational changes and inactivates the RNA-dependent and DNA-dependent DNA polymerase activities by causing a disruption of the enzyme's catalytic site. Resistance develops rapidly in cells exposed to Nevirapine. High resistance is associated with mutations at reverse transcriptase codons 101,103,106.108,135,181,188 and 190.

Pharmacokinetics

Absorption:

Nevirapine is absorbed rapidly after oral doses and absorption is not affected by food. Peak plasma concentrations occurs 4 hours after a single dose. Oral bio availability is greater than 90%

Distribution:

Nevirapine crosses the blood-brain barrier with a ratio of cerebrospinal fluid to serum concentrations of about 0.45. Binding to plasma protein is reported to be up to 36%. Nevirapine crosses the placenta and is distributed into breast milk. Nevirapine is about 60% bound to plasma proteins.

Metabolism:

Nevirapine extensively metabolized by hepatic microsomal enzymes, principally by cytochrome P450 iso enzymes of CYP3A family and produces several metabolites including 2-, 3-, 8-, and 12- hydroxy nevirapine. Auto induction of these enzymes results in a 1.5 to 2 fold increase in apparent clearance after 2 to 4 weeks of administration of usual doses, and decrease in terminal half life from 45 hours to 25-30 hours over the same period.

Elimination:

Nevirapine is mainly excreted in urine as glucuronide conjugates of the hydroxylated metabolites.

Bioavailability: 90%

Half-life elimination: 25 to 30 hours

Excretion: Urine

Drug interactions

Nevirapine induces CYP3A4 enzyme, co administration with agents metabolized by this system may lower the plasma levels. Methadone withdrawal has been reported in patients receiving Nevirapine. Rifampicin and ketoconazole are contraindicated in patients receiving nevirapine. Plasma ethylestradiol levels decrease significantly with nevirapine coadministration. Although Nevirapine can lower plasma concentrations of protease inhibitors, most such combinations do not require dose adjustment.

Adverse reactions

The most common adverse effect of Nevirapine is skin rash, usually occurring within first 6 weeks of starting therapy. Severe and life-threatening skin reactions have occurred, including stevens-johnson syndrome, and rarely, toxic epidermal necrolysis. Hypersensitivity reactions including angioedema, utricaria, and anaphylaxis have been reported. Severe hepatotoxicity, including hepatitis and hepatic necrosis, occasionally fatal, has occurred and may be more prevalent in women and patients with high CD4 cell counts at the start of treatment. Other common adverse effects include nausea, vomiting, diarrhea, abdominal pain, fatigue, drowsiness and head ache.

Clinical uses

Nevirapine is FDA-approved drug for treating HIV infection in adults and children, in combination with other antiretroviral agents. It can be very effective during long-term administration in multi drug regimens.

Dosage and administration:

HIVinfection
Adult: 200mg once daily for first 14 days, then increased to 200mg twice daily provided that no rash is present

Child: 2months- 8 years: 4mg/kg once daily for 14 days and then if no rash is present, 7mg/kg twice daily.

8 to 16 years: 4mg/kg once daily for 14 days and then 4mg/kg twice daily thereafter.

4.2 POLYMER PROFILE

Human serum albumin[53,54,55,56,57]

Synonym: human serum albumin; HSA

Structure:

The molecular weight of HSA has frequently been cited as 66,120 or 66,267, but it was revised in 1990to 66,430. All three values are based on amino acid sequence information. HSA is a single polypeptide chain consisting of about 583 amino acid residues and no carbohydrates. At pH 5-7 it contains 17 intra chain disulfide bridges and 1 sulfahydryl group.

Physicl properties:

Appearance	: powder –white to light tan
Solutions	: clear to slightly hazy and amber
PI in water at 25^0 c	: endogenous material
Fatty Acid Depleted	: 5.3
pH of 1% Solution	: 5.2-7
Intrinsic viscosity,	: 0.0413
Stability/ storage stable	: if stored at 2-8^0 c, HSA powders and HSA solutions were for a minimum of 2.5 years

Solubility/ solution stability:

Albumins are readily soluble in water and can only be precipitated by high concentrations of neutral salts such as ammonium sulfate. The solubility of powdered HSA in deionised water at 40 mg/ml and obtains clear to very slightly hazy, faint yellow solutions. The solution stability of HAS is very good (especially if the solutions are stored as frozen laliquots). However albumin is readily coagulated by heat. When heated to 50^0c or above, albumin quite rapidly forms hydrophobic aggregates, which do not revert to monomers upon

cooling. At somewhat lower temperatures aggregation is also expected to occur, but at relatively slower rates.

Product description / usage:

Albumins are a group of acidic proteins, which occur plentifully in the body fluids, and tissues of mammals and in some plant seeds. Unlike globulins, albumins have comparatively low molecular weights, are soluble in water, are easily crystallized, and contain an excess of acidic amino acids. Serum and plasma albumin is carbohydrate- free and comprises 55-62% of the protein present.

Albumin binds water, Ca^{2+} ,Na^{+} and K^{+} due to a hydrophobic cleft, albumin binds fatty acids, bilirubin, hormones and drugs. The main biological function of albumin is to regulate the colloidal osmotic pressure of blood. Human and bovine albumins contains 16%nitrogen and are often used as standards in protein calibration studies. Albumin is used solubilise liquids, and is also used as a blocking agent in westeron blots or ELISA applications. Globulin free albumins are suitable for use in applications where no other proteins should present (e.g.,electrophoresis).

Applications :

- Antibody purification
- Binding and transport studies
- Blood banking reagents
- Culture media (microbial)
- Cell culture (general)
- Electrophoresis(M.W.standard)
- ELISA (blocking reagent)
- ELISA (non specific binding)
- Enzyme systems
- Hapten carrier
- Immunocytochemistry
- Immunohematology
- Mitogenic assays

- Molecular biology
- Protein base or filler
- Protein supplement (controls)
- Protein standard (M.W.,amino acids)
- RIA systems
- seriology

5.0 AIM AND SCOPE OF THE STUDY

Nevirapine (NVP), a non-nucleoside reverse transcriptase inhibitor, is one of the most prescribed antiretroviral drugs for reducing the morbidity and mortality related to the infection of human immunodeficiency virus (HIV). NVP has been widely applied as a prophylaxis dose against mother-to-child HIV transmission in developing countries .In combination therapy, NVP was used in the initial regimen to reduce impacts on the clinical implications of drug resistance. Nevirapine is currently available in two dosage forms namely tablets and suspensions. commercially nevirapine is highly hydrophobic and very poorly water soluble. Nevirapine is a BCS class II drug and therefore poses a challenge in the design of dosage form due to its low aqueous solubility. Besides its poor solubility may also aspects the bioavailability of the drug.

Albumin is an attractive macromolecular carrier and widely used to prepare nanospheres and nanocapsules, due to its availability in pure form and its biodegradability, nontoxicity and non immmunogenicity. Both Bovine Serum Albumin or BSA and Human Serum Albumin or HSA have been used. As a major plasma protein, albumin has a distinct edge over other materials for nanoparticle preparation. On the other hand, albumin nanoparticles are biodegradable, easy to prepare in defined sizes, and carry reactive groups (thiol, amino, and carboxylic groups) on their surfaces that can be used for ligand binding and/or other surface modifications and Based on albumin nanoparticles offer the advantage that ligands can easily be attached by covalent linkage. Drugs entrapped in albumin nanoparticles can be digested by proteases and drug loading can be quantified.

Based on the above considerations the present study proposed to develop nevirapine nanoparticles using human serum albumin with different polymer ratios and to evaluate the nanoparticles for physico-chemical, in-vitro, in-vivo release characterstics. The findings of the polymer may help design a better and controlled release system with improved bioavailability of nevirapine.

The following parameters were examined

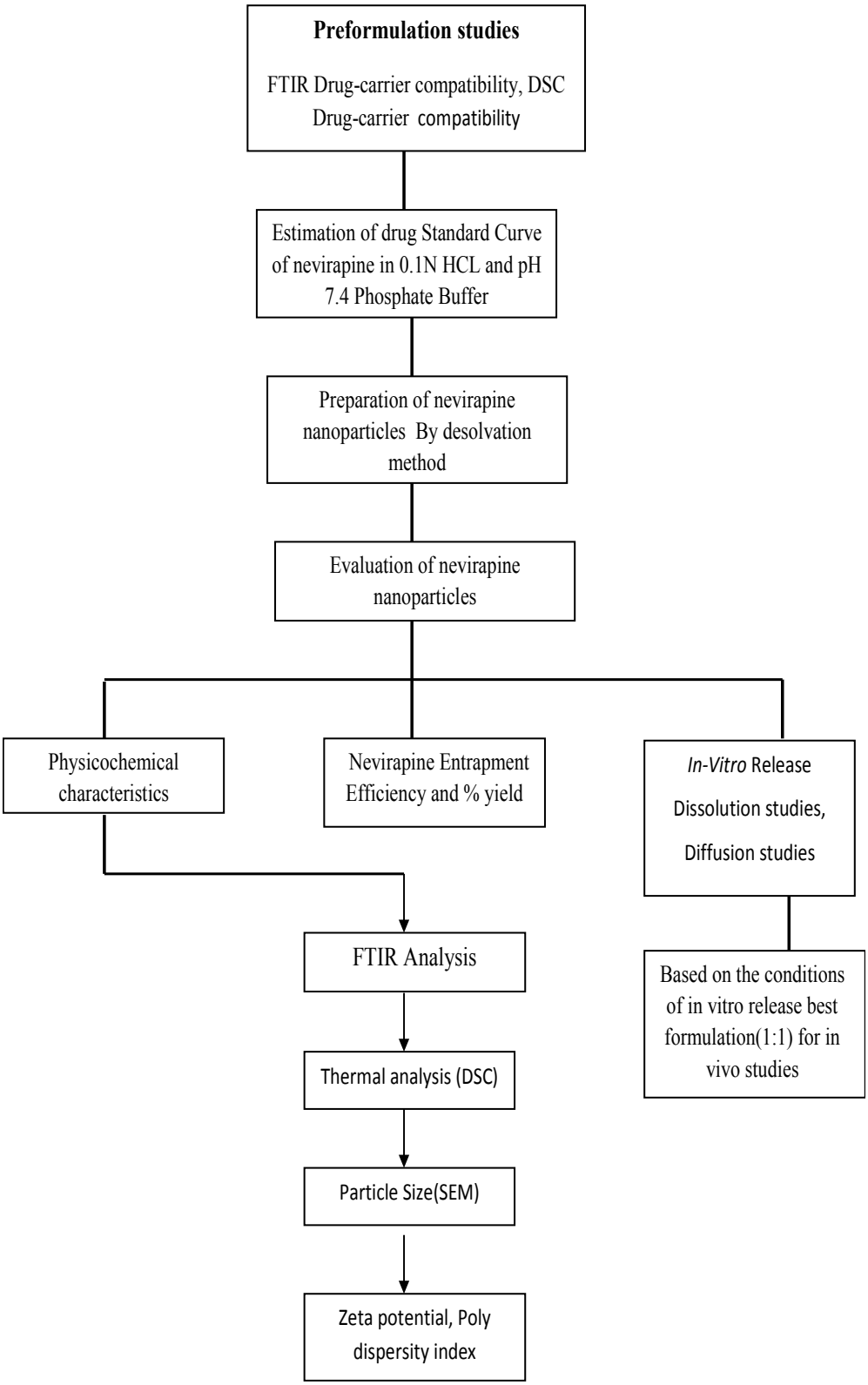
1. Pre-formulation studies.
    a) Drug and carrier interaction by FT-IR spectroscopy.
    b) Drug and carrier interaction by Differential Scanning Calorimetry(DSC)
2. Preparation of standard curve of Nevirapine in pH 1.2 and 7.4 Phosphate Buffer
3. Preparation of Nevirapine loaded albumin nanoparticles by Desolvation method.
4. Evaluation of Nevirapine loaded albumin nanoparticles
    a) Drug and carrier interaction by FT-IR spectroscopy.
    b) Particle size determination by Scanning Electron Microscopy(SEM)
    c) Surface characteristic by Zeta potential analyzer
    d) Thermal analysis by Differential Scanning Calorimetry (DSC).
    e) Nevirapine encapsulation efficiency and percentage yield of the nanoparticles.
    f) Invitro release of nevirapine from the nanoparticles.

        Dissolution studies

        Diffusion studies

Based on the conditions of in vitro release best sample was used for in vivo studies in animal model.

6.0 PLAN OF WORK



7.0 MATERIALS

Drugs and Chemicals

Drugs and Chemicals - Supplier/ Manufacturer

Nevirapine	- Ranbaxy labs Pvt. Ltd .Mumbai.
Human serum albumin	- (Sisco Research Lab. Bombay)
25%glutaraldehyde	- (Loba Chemie Bombay)
HPLC water	- Merck Pvt, Ltd Mumbai.
99% Absolute Alcohol (Changshu Yangyuan Chemical, China),	
Sodium Hydroxide	- Spectrum Reagents & Chemicals, Cochin
Potassium Dihydrogen Phosphate	- Spectrum Reagents & Chemicals, Cochin
Sodium Tri Poly Phosphate	- S.D Fine chem. Ltd, Mumbai
Dialysis membrane 110	- Himedia Laboratory, Mumbai
Programmable Dissolution Apparatus	-Veego (Mumbai)

7.1 INSTRUMENTS

Instruments - Model/ Manufacturer

FT-IR spectrophotometer	- Perkin Elmer Spectrum RX 1
Scanning electron microscope	- Joel model JSM 6400, Tokyo
UV-Visible Spectrophotometer	- Perkin Elmer Lambda 25
Single pan digital balance	- Shimadzu BL220H
Microscope - Unilab	
Digital pH meter	- Hanna instruments, Italy HI98
Magnetic stirrer	- Eltek MS 2012.
Sonicater - Bandelin Sono plus Model HD , 2070	
Freeze Drier	- Labconico, USA
Research centrifuge - Hitachi Centrifuge USA	
Differential Scanning Calorimetry	- DSC DA 60 Shimadzu.Japan
Zeta potential analyzer	- Zetasizer 3000HS,Malvern instrument, UK.

8.0 METHODS

8.1 PREFORMULATION STUDIES [58,59]

Before formulation of drug substances into a dosage form, it is essential that the drug and polymer should be chemically and physically characterized. Preformulation studies give the information needed to define the nature of the drug substance and provide a framework for the drug combination with pharmaceutical excipients in the fabrication of a dosage form.

Fourier Transform Infra Red Spectroscopy (FTIR)

Compatibility study of drug with the polymer was determined by FTIR Spectroscopy using Perkin Elmer RX1. The pellets were prepared by gently mixing of 1mg sample with 200mg potassium bromide at high compaction pressure. The scanning range was 450 to 4000 cm^{-1} and the revolution was 4 cm^{-1}. The pellets thus prepared were examined and the spectra of drug and the polymer in the formulations were compared with that of pure drug or polymer spectra.

Differential Scanning Calorimetry (DSC)

Differential scanning calorimetric curve of pure nevirapine, human serum albumin (HSA) polymer and mixture of drug and polymer measurement were carried out by using a thermal analysis instrument equipped with a liquid nitrogen sub ambient accessory. 2-6mg samples were accurately weighed in aluminum pans thematically sealed and heated at a rate of 10ºC per min -1 in a 30 to 300 ºC temperature under nitrogen flow of 40 ml / min.

8.2 Construction of standard curve for Nevirapine [60]

Nevirapine can be estimated spectrophotometrically at 283nm as it obeys Beer's-Lambert's law limit in the range of 2-20 µg/ml.

Preparation of reagents

Preparation of 0.1 N HCl

8.5 ml of concentrated HCl was dissolved in 1000 ml of distilled water.

Stock solution

100 mg of Nevirapine was dissolved in 100 ml of 0.1 N HCl, to get a solution of 1000 µg/ml concentration.

Standard solution

10 ml of stock solution was made to 100 ml with 0.1 N HCl thus giving a concentration of 100 µg/ml. Aliquot of standard drug solution ranging from 0.5 ml, 1 ml, 1.5 ml, 2 ml and 2.5 ml were transferred into 10 ml volumetric flask and were diluted up to the mark with 0.1 N HCl. Thus the final concentration ranges from 2 – 20 µg. Absorbance of each solution was measured at 283 nm against 0.1 N HCl as a blank. A plot of concentrations of drug Vs absorbance was plotted.

Preparation of Phosphate buffer pH 7.4

50 ml of potassium Dihydrogen phosphate was placed in a 200ml volumetric flask and added 39.1 ml of 0.2m sodium hydroxide and then distilled water to make up to 200ml.

Preparation of 0.2m potassium Dihydrogen phosphate

27.218g of potassium Dihydrogen phosphate was dissolved in distilled water and made up to 1000 ml.

Preparation of 0.2m sodium hydroxide

8g of sodium hydroxide was dissolved in distilled water and made up to 1000 ml.

Preparation of standard drug solution

Stock Solution

100mg of Nevirapine was dissolved in 100ml of Phosphate buffer saline pH 7.4 so as to get a stock solution of 1000 µg/ml concentration.

Standard Solution

2 ml of stock solution was diluted to 100ml with pH 7.4 phosphate buffer thus giving a concentration of 20 μg/ml of the drug .Aliquot of standard drug solution ranging from 1ml to 9ml were transferred 10ml volumetric flask and were diluted up to the mark with pH 7.4 phosphate buffer. Thus the final concentration ranges from 2-20 μg/ml as per Beer Lambert's law. Absorbance of each solution was measured at 283.0 nm against phosphate buffer pH 7.4 as a blank and the concentration of drug Vs absorbance was plotted.

Standard curve of Nevirapine

The standard curve of Nevirapine was determined in 0.1 N HCL (pH 1.2) by using UV-Visible spectrophotometer at 283 nm. Graph was plotted by taking absorbance (nm) on X-axis verses concentration (µg/ml) on Y-axis and it is follows the Beer's law. The results were shown in table 4.

Standard curve of Nevirapine in 0.1 N Hcl

Concentration (µg/ml)	Absorbance (at 283nm)
0	0
2	0.113
4	0.234
6	0.382
8	0.516
10	0.652
12	0.723
Slope	0.026
R^2	0.998

Table 4 standard curve of nevirapine pH 1.2

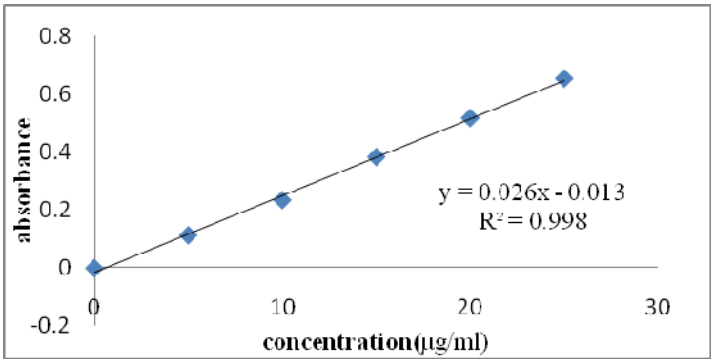


Fig 2 standard curve of nevirapine pH 1.2

Preparation of standard graph of Nevirapine in pH 7.4 phosphate buffer

Standard graph for the drug nevirapine was done in pH 7.4 phosphate buffer.Table 5 shows the concentrations of nevirapine in pH 7.4 phosphate buffer and the respective absorbances. It shows the standard graph of nevirapine in 7.4 phosphate buffer.

Spectrophotometric data for standard curve of Nevirapine in pH 7.4 phosphate buffer

Concentration (µg/ml)	Absorbance (at 283nm)
0	0
2	0.089
4	0.187
6	0.286
8	0.369
10	0.458
12	0.540
Slope	0.045
R^2	0.999

Table 5 standard curve of nevirapine pH 7.4

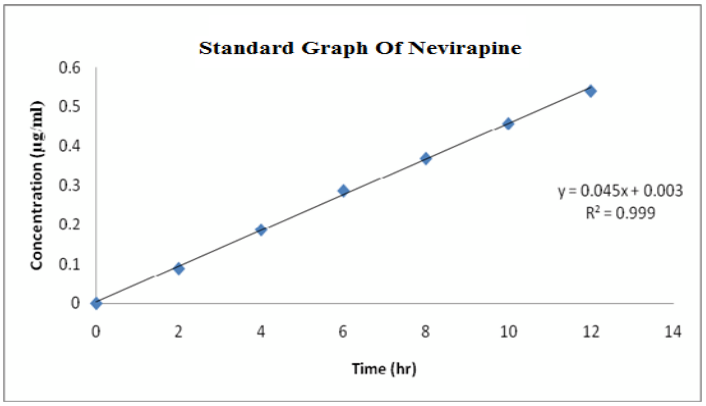


Fig 3 standard curve of nevirapine pH 7.4

8.3 PREPARATION OF NEVIRAPINE LOADED HUMAN SERUM ALBUMIN (HSA) NANOPARTICLES[61]

The formula of nevirapine nanoparticles is given in table no-6 . Nevirapine nanoparticles were prepared by desolvation technique. Human serum albumin (HSA) nanoparticles were prepared by a desolvation technique as described previously (Marty et al 1978). In this method 200mg of drug was dissolved in 100ml of ethanol. The drug containing ethanol solution was dropwise introduced into the different concentrations of human serum albumin solution (200mg,400mg,800mg).To the above mixture 25%glutaraldehyde solution was added and the mixture was continuously stirred for 2 hrs followed by sonication for 5mins. The obtained nanoparticles were seperated by cold centrifuged at 12000rpm in a glucose bed for 30mins using Hitachi centrifuge. The supernetantent liquid was analysed by spectrophotometer to caliculate the percentage drug entrapment and drug loading. The final suspension was then frozen and lyophilized at 0.4 mbar and -40^{o}C for 5 hrs using glucose and lactose (1:2) as cryoprotective agents. The lyophilised nanoparticles were stored in a desicator at 4^{0}C.

Table 6 Nevirapine loaded HSA nanoparticles

Formulation code	Drug	Polymer	Drug polymer ratio	Sonication time
F1	200mg	200mg	1:1	5mins
F2	200mg	400mg	1:2	5mins
F3	200mg	800mg	1:4	5mins

8.4 EVALUATION OF NANOPARTICLES

8.4.1 Drug and carrier interaction by Fourier Transform Infra Red Spectroscopy

Compatibility study of drug with the polymer was determined by FTIR Spectroscopy using Perkin Elmer RX1. The pellets were prepared by gently mixing of 1mg sample with 200mg potassium bromide at high compaction pressure. The scanning range was 450 to 4000 cm^{-1} and the revolution was 4 cm^{-1}.

8.4.2 Particle size determination by Scanning Electron Microscopy [62]

The size of the nanoparticles was analyzed by scanning electron microscope .The instrument used for this determination was JEOL MODEL JSM 6400 scanning electron microscope (SEM). The nanoparticles were mounted directly on the SEM stub, using double –sided, sticking tape and coated with platinum and scanned in a high vacuum chamber with a focused electron beam. Secondary electrons, emitted from the samples were detected and the image formed.

Surface Characteristics by Zeta Potential

The Zeta potential of nanoparticles was measured on a zeta potential analyzer Zetasizer 3000 HS Malvern instrument. The samples were diluted with pH 7.4 and placed in eletrophoretic cell and measured in the automatic mode.

8.4.3 Thermal Analysis by Differential Scanning Calorimetry (DSC)

Differential scanning calorimetric measurement of nevirapine, human serum albumin nanoparticles was carried out by using a thermal analysis instrument equipped with a liquid nitrogen sub ambient accessory. 2-6mg samples were accurately weighed in aluminum pans thermatically sealed and heated at a rate of 10ºC per min -1 in a 30 to 300ºC temperature under nitrogen flow of 40 ml / min.

8.4.4 Encapsulation efficiency and Percentage yield of the nanoparticles [63]

The Encapsulation efficiency and percentage yield of the nanoparticles were determined by the separation of nanoparticles from the aqueous medium containing non associated nevirapine by cold centrifugation at 12000g for 30 minutes.The amount of free nevirapine in the supernatant was measured by UV method at 283 nm. The nevirapine encapsulation efficiency (EE) and percentage yield of the nanoparticles was calculated as follows.

$$\text{Encapsulation efficiency} = \frac{\text{Total amount of Nevirapine} - \text{Free Nevirapine}}{\text{Weight of nanoparticles}} \times 100$$

$$\text{Percentage yield} = \frac{\text{Total amount of Nevirapine} - \text{Free Nevirapine}}{\text{Total amount of Nevirapine}} \times 100$$

8.4.5 *Invitro* release of nevirapine from the nanoparticles

Dissolution studies

The dissolution profiles of the plain drug and the nanoparticle formulations were determined in USP dissolution apparatus – II using 900ml of pH – 1.2 buffer for the first 2 hrs and continued with Phosphate buffer pH – 7.4. The dissolution media were maintained at 37°±0.5°C with a paddle rotation speed at 50 rpm. The amount of drug used was equivalent to 2 mg. At specified time intervals (0.5,1,2,4,6,8,10,12,and24hrs) 5ml of dissolution media were withdrawn and replaced with an equal volume of the fresh medium to maintain constant volume of the media. Samples were filtered through a 0.22µm nylon membrane filter (Millipore, Bedford, MA) and assayed for drug content spectrophotometrically at 283nm using UV/Vis double beam spectrophotometer after appropriate dilution with the corresponding media. Cumulative percentage of drug dissolved in the preparations was calculated using calibration equations. Dissolution tests were performed in triplicate for each formulation (n = 3).[64]

8.4.6 Diffusion studies

The nevirapine loaded human serum albumin (HSA) nanoparticles were separated from the aqueous suspension medium through ultracentrifugation. Nanoparticles equivalent to 2mg of nevirapine nanoparticles were redispersed in 10ml phosphate buffer solution pH-7.4 and placed in a dialysis membrane bag with a molecular cut-off of 5 kDa which acts as a donor compartment, tied and placed into 10 ml 7.4 phosphate buffer solution in a beaker which acts as a receptor compartment .The entire system was kept at 37ºC with continuous magnetic stirring. At appropriate time intervals (1, 2, 3, 4,6,8,10,12,14,16,18,20,22 and 24 hrs), 1 ml of the release medium was removed and 1 ml fresh 7.4 phosphate buffer solution

was added in to the system. The amount of nevirapine in the release medium was evaluated by UV-Visible Spectrophotometer at 283 nm.

8.4.7 Kinetics of drug release[65,66]

In order to understand the mechanism and kinetic of drug release, the drug release data of the in-vitro dissolution study were analyzed with various kinetic models like zero order, first order, Higuchi's, Peppa's and Coefficient of correlation (r) values were calculated for the linear curves by regression analysis of the above plots.

I) Fitting of Results into Different Kinetic Equations

The results of in vitro release profile obtained for all the formulations were plotted in modes of data treatment as follows: -

1. Zero - order kinetic model - Cumulative % drug released versus time.
2. First – order kinetic model - Log cumulative percent drug remaining versus time.
3. Higuchi's model - Cumulative percent drug released versus square root of time.
4. Korsmeyer equation / Peppa's model - Log cumulative percent drug released versus log time.

A) Zero order kinetics

Zero order release would be predicted by the following equation

$$A_t = A_0 - K_0 t$$

Where,

A_t = Drug release at time't'.

A_0 = Initial drug concentration.

K_0 = Zero - order rate constant (hr^{-1}).

When the data plotted as cumulative percent drug release versus time, and the plot is linear, and then the data obeys Zero – order equal to K_0.

B) First Order Kinetics

First – order release would be predicted by the following equation:-

$$\text{Log } C = \log C_0 - Kt / 2.303$$

Where, C = Amount of drug remained at time‘t’.

C_0 = Initial amount of drug.

K = First – order rate constant (hr^{-1}).

When the data plotted as log cumulative percent drug remaining versus time yields a straight line, then the release follow first order kinetics. The constant ‘K’ can be obtained by multiplying 2.303 with the slope values.

C) Higuchi’s model

Drug release from the matrix devices by diffusion has been described by following Higuchi’s classical diffusion equation

$$Q = [D\varepsilon / \tau (2\ A - \varepsilon Cs)\ Cst]^{½}$$

Where,

Q = Amount of drug released at time‘t’.

D = Diffusion coefficient of the drug in the matrix.

A = Total amount of drug in unit volume of matrix.

Cs = the solubility of the drug in the matrix.

ε = Porosity of the matrix.

τ = Tortuosity.

t = Time (hrs) at which ‘q’ amount of drug is released.

Above equation may be simplified if one assumes that ‘D’, ‘Cs’ and ‘A’ are constant. Then equation becomes

$$Q = Kt^{1/2}$$

When the data plotted according to equation i.e. cumulative drug release versus square root of time yields a straight line, indicating that the drug was released by diffusion mechanism. The slope is equal to ‘K’ (Higuchi’s 1963).

D) Korsmeyer equation / Peppa’s model

To study the mechanism of drug release from the sustained-release of acyclovir nanoparticles, the release data were also fitted to the well-known exponential equation (Korsmeyer equation/ peppa’s law equation), which is often used to describe the drug release behavior from polymeric systems.

$$M_t / M_\alpha = Kt^n$$

Where, M_t / M_α = the fraction of drug released at time‘t’.

K = Constant incorporating the structural and geometrical characteristics of the drug / polymer system.

n = Diffusion exponent related to the mechanism of the release.

Above equation can be simplified by applying log on both sides,

And we get: - $\text{Log } M_t / M_\alpha = \text{Log } K + n \text{ Log } t$

When the data plotted as log of drug released versus log time, yields a straight line with a slope equal to ‘n’ and the ‘K’ can be obtained from y – intercept. For Fickian release ‘n’ = 0.5 while for anomalous (non - Fickian) transport ‘n’ ranges between 0.5 and 1.0.

Table 7 : Mechanism of Drug Release as per Korsmeyer Equation / Peppa's Model

S. No.	n Value	Drug release
1.	0.50	Fickian release
2.	0.5 < n <1.0	Non – Fickian release
3.	1.0	Class II transport

E). Statistical analysis

The release data were subjected to ANOVA with Tukey-Kramer multiple comparision test.

8.5 *In-vivo* Evaluation studies:

Animals: New Zealand white rabbits weighing 1.5 to 2.5kg were obtained from Swamy Vivekananda College of pharmacy animal house and used in this study. The animals were fed with cabbage and water. They were maintained in standard laboratory conditions at 21±2 ^{0}C and relative humidity of 55-60%. The animals were fasted overnight before the experiment. The study protocol was approved by the Institutional Animal Ethical Committee and the protocol number is SVCP/IAEC/M.Pharm/08/2013.

Requirements: Cotton
Surgical blade
26G needle
Blood collecting tubes (EDTA tubes)
Plasma sample collecting tubes

Sex: Male

No. of animals: 06

Animal dose:

Nevirapine: 20mg/kg
Nevirapine nanoparticles:12.5mg/kg

Procedure for collection of blood:

Collection of blood from marginal ear vein:

The animal was placed in a restainer. Hair of the ear was shaved smoothly with blade without disturbing the blood vessels. Ear was cleaned with 95% v/v alcohol on the collection site and rapid rubbing on the ear to dilate blood vessels which is easy to collect the blood. 2G needle was inserted in vein to collect the blood from marginal ear vein. After collecting blood, clean sterile cotton was kept on the collection site and finger pressure was applied to stop the bleeding.[69]

Experimental procedure: Rabbits were classified into 2 different groups each group consisting of 6 animals

GROUP I	Nevirapine
GROUP II	Nevirapine nanoparticals

Procedure : After overnight fasting, Group1, and Group 2, animals received Niverapine (20mg/kg), Niverapine nanoparticals (12.5mg/kg) respectively through an intragastric tube. Blood samples (1 ml) were collected in heparinized tubes from the marginal ear vein at 0, 0.5, 1.5, 2, 2.5, 3, 3.5, 4, 5, 6, 8, 10, 12 and 24 h after drug administration and plasma was separated by using centrifugation and stored at -20°C. Samples were analysed by validated high performance liquid chromatography (HPLC).[68]

Bioanalytical work:

Extraction of nevirapine and nevirapine nanoparticle from plasma:

An aliquot of 500µl of plasma samples was pipetted into an eppendrof's tube of 1.5ml capacity. 500µl ethyl acetate was added, vortexed for 3 minute and centrifuged at 10,000 rpm for 15min. Then 300 µl of the supernatant was taken into another microcentrifuge tube and vaccum dried in centrivac. The residue obtained was reconstituted in 100µl of mobile phase. Plasma was filtered through 0.22 µm membrane (13 mm) and 20 µl volumes was injected. All the two analytes; NVP and NVP NP were detected at 283nm[70]

Hplc analysis:

Preparation of mobile phase:

Potassium dihydrogen phosphate buffer was weighed (131.6g) and dissolved in 1000ml of water to get 1M solution. From this solution 15ml was taken and volume was made up to 100ml to get a solution of 15mM. The pH of the solution was adjusted to 3.2 with ortho phosphoric acid. The final volume was adjusted with acetonitrile, which resulted in pH 3.5 for final mobile phase. The final solution was mixed well and sonicated for 10 min and filtered using whatman filter paper.

Chromatographic condition:

A water reverse phase C-18 column, equilibrated with mobile phase 15mM aqueous phosphate buffer: acetonitrile was used. The active principle was eluted isocratically and the mobile phase flow rate maintained at 1.0 mi/min. the effluents were mentioned at 283 nm with the detector. The sample was injected using a 20µl fixed loop, and the total run time was 10min.

Extraction efficiency:

The extraction efficiency was calculated by comparing the peak heights of nevirapine spiked-pooled blank plasma samples with that of respective standard nevirapine samples

8.5.1 Pharmacokinetic parameters[71]

The pharmacokinetic parameters were calculated for each rabbit of group I, group II, by the semi logarithmic plot of plasma nevirapine concentration at various intervals. The following pharmacokinetic parameters were calculated:

1. Elimination rate constant (K_e): The elimination rate constant was determined using the formula

   K_e = -2.303 x slope of extrapolated curve

2. Elimination half life ($t_{1/2}$): $t_{1/2}$ was calculated using the formula

   $t_{1/2} = 0.693/K_e$

3. Absorption rate constant (K_a): This was determined by the method of residuals. The log linear portion of the decline phase was back extrapolated for each curve. The plasma concentration along this extrapolated line was C. the observed plasma concentration C was subtracted from the corresponding extrapolated value at each time point. The semi logarithmic plot of residuals (C-C) against time yields a straight line.

   K_a = -2.303 x slope of residual line

4. Absorption half life**:** It was calculated using the formula

   $T_{1/2(a)} = 0.693/K_a$

5. Apparent volume of distribution (V_d): It was calculated using the formula

$$V_d = \frac{K_a F X_0}{(K_a - K_e) \text{ y intercept}}$$

6. Time to C_{max} (t_{max}): t_{max} was calculated using the formula

$$t_{max} = \frac{\ln K_a - \ln K_e}{K_a - K_e}$$

7. Maximum plasma concentration (C_{max}): C_{max} was calculated using the formula

$$C_{max} = \text{Y intercept } (e^{-Ke.\ Tmax} - e^{-Ka.\ Tmax})$$

8. Area under curve (AUC_{0-12}): AUC_{0-12} was calculated using the formula

$$AUC = \frac{F\ X_0}{V_d.K_e}$$

9. $AUC_{0-\infty}$ was calculated using the formula

$$AUC_{0-\infty} = \frac{C_0}{K_e}$$

STATISTICS:

The values are expressed in mean ± SEM. One way ANOVA followed by Tukey's multiple comparison Test was used to analyse the effect of different doses of silymarin when compared to control, with the help of Graph Pad Instat software, version 3.01. $P<0.05$ considered as significant.

9.0 RESULTS

9.1 COMPATIBILITY STUDIES

A. Drug and carrier intereaction by FT-IR Spectroscopy

FT-IR spectra of nevirapine, human serum albumin in physical mixture of nevirapine and HSA and nevirapine loaded albumin nanoparticles are shown in tables8,9,10 and 11 and figures 4,5,6 and 7. Nevirapine showed from characterstic peaks at 2885.6 cm^{-1} (C-H-alkyl strecting),1699.34 cm^{-1}(C=O-aromatic ketones), 1651.34cm^{-1}(COOH unsaturated carboxylic acid stretching),3043.77cm^{-1}(O-H carboxylic acid-stretching). All these characteristic peaks of nevirapine were present in the spectra of either physical mixture or nanoparticles thus confirming compatability of the drug with polymer

Sl.no	IR Spectrum	Peaks(cm^{-1})	Groups	Stretching /Deformation
1	Nevirapine	2885.6	C-H(alkyl)	Stretching
		1699.34	C=O(aromatic ketone)	Stretching
		1651.12	COOH(unsaturated carboxylic acid)	Stretching
		3043.77	O-H(Carboxylic acid)	Stretching

Table 8 IR SPECTRUM OF NEVIRAPINE

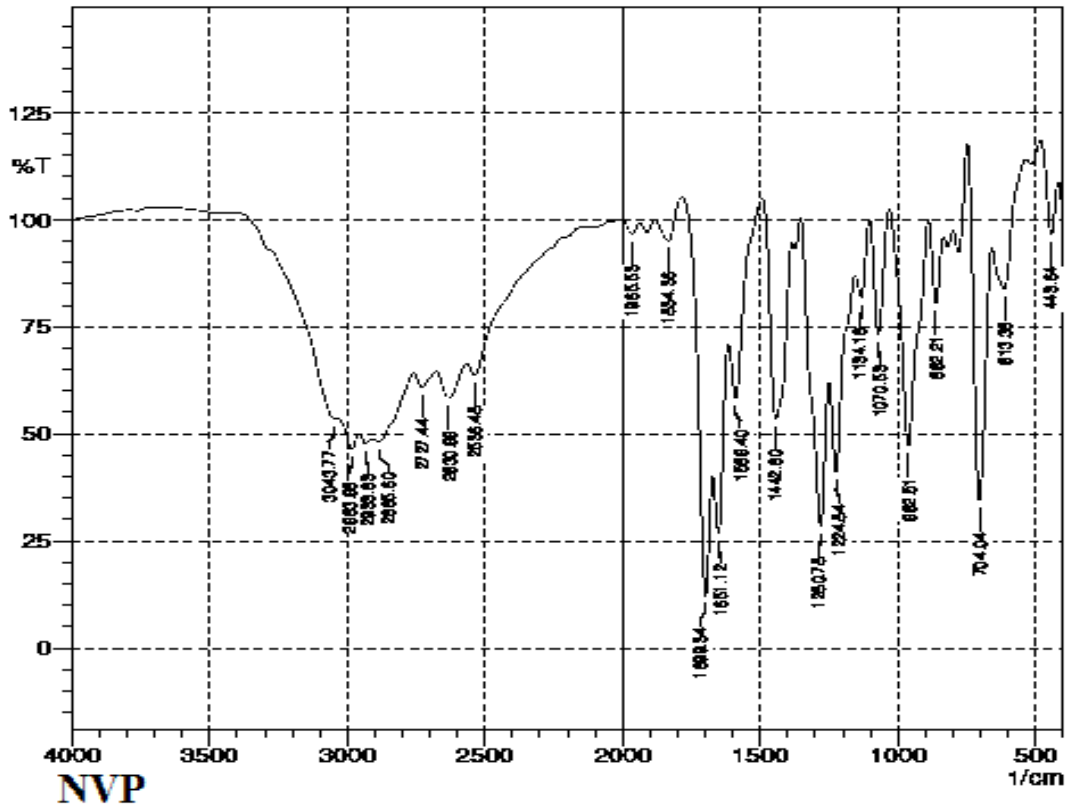


Fig 4 IR SPECTRUM OF NEVIRAPINE

2	Blank nanoparticles of Human serum albumin	2872.1	C-H(alkyl)	Stretching
		1653.05	C=O(aromatic ketone)	Stretching
		1629.6	COOH(unsaturated carboxylic acid)	Stretching
		3084.28	O-H(Carboxylic acid)	Stretching

Table 9 IR SPECTRUM OF BLANK NANOPARTICLES USING HSA

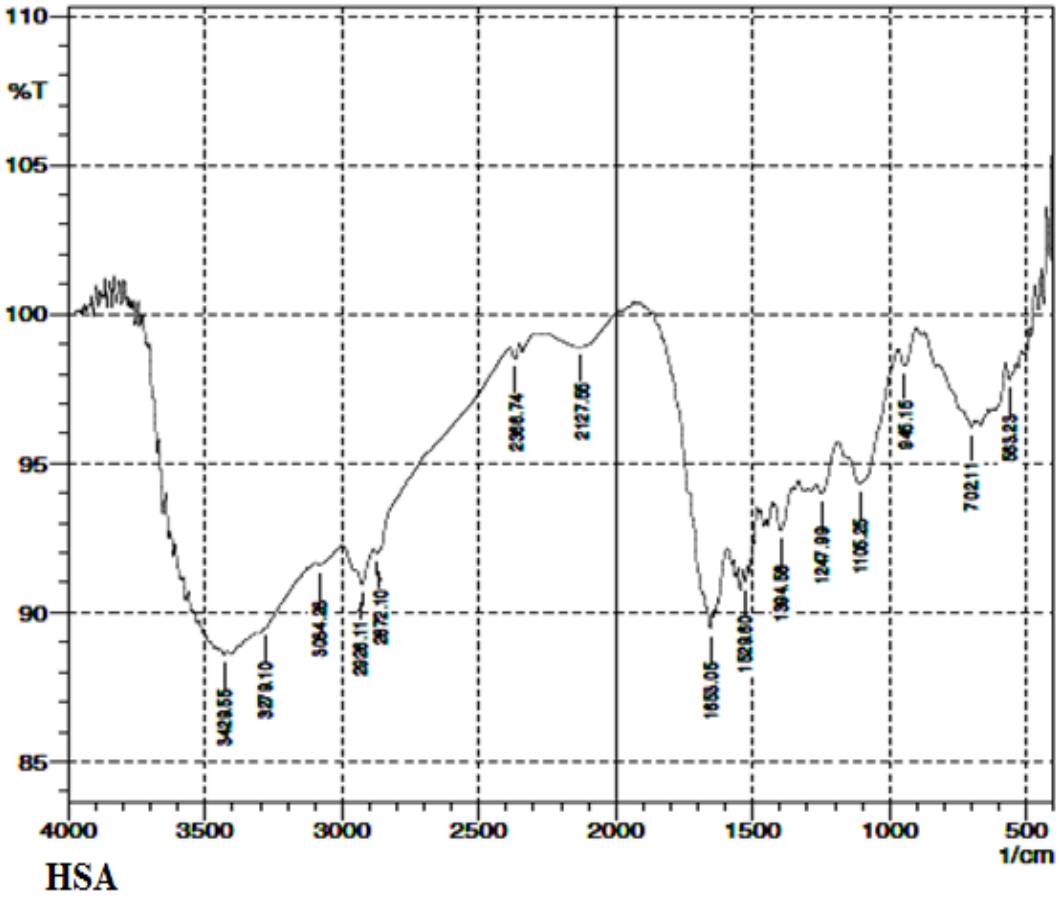


Fig 5 IR SPECTRUM OF BLANK NANOPARTICLES USING HSA

3	Physical mixture of Nevirapine and Human serum albumin	2891.39	C-H(alkyl)	Stretching
		1697.41	C=O(aromatic ketone)	Stretching
		1651.12	COOH(unsaturated carboxylic acid)	Stretching
		3045.7	O-H(Carboxylic acid)	Stretching

Table 10 IR SPECTRUM OF PHYSICAL MIXTURE OF NEVIRAPINE WITH HSA

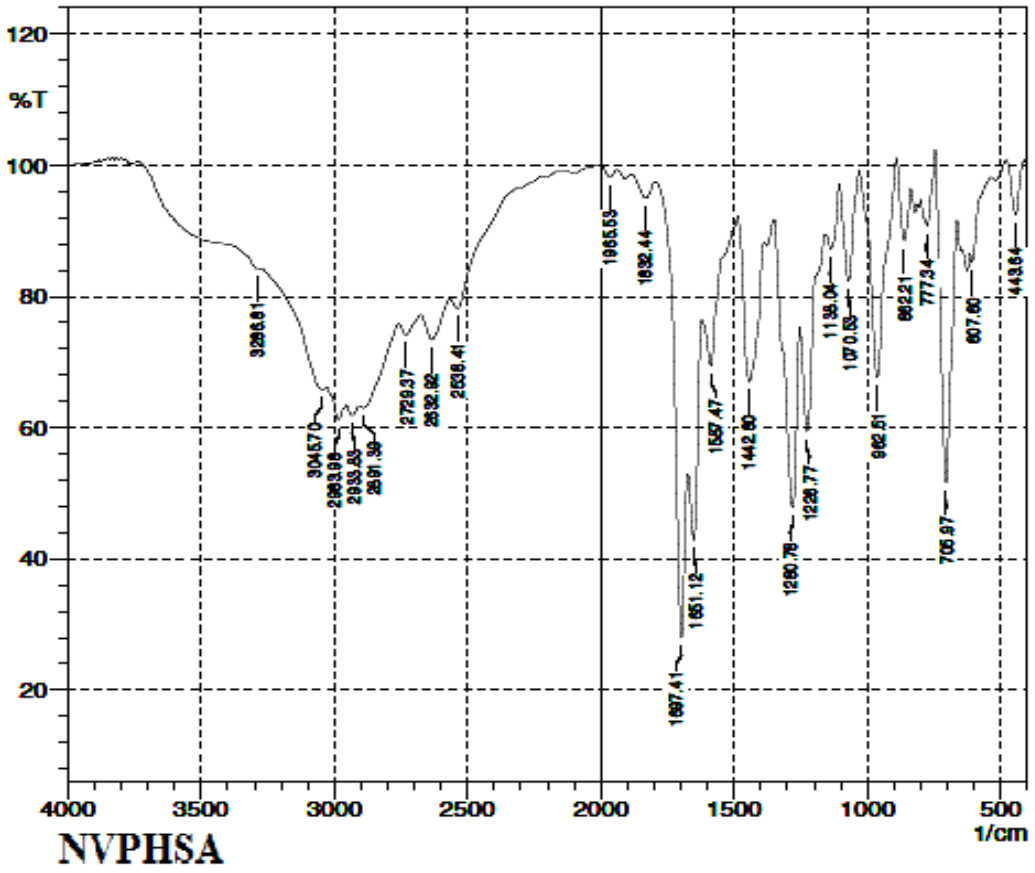


Fig 6 IR SPECTRUM OF PHYSICAL MIXTURE OF NEVIRAPINE WITH HSA

4	Nevirapine nanoparticles of Human serum albumin	2856.67	C-H(alkyl)	Stretching
		1654.98	C=O(aromatic ketone)	Stretching
		1645.03	COOH(unsaturated carboxylic acid)	Stretching
		2926.11	O-H(Carboxylic acid)	Stretching

Table 11 IR SPECTRUM OF NEVIRAPINE NANOPARTICLES

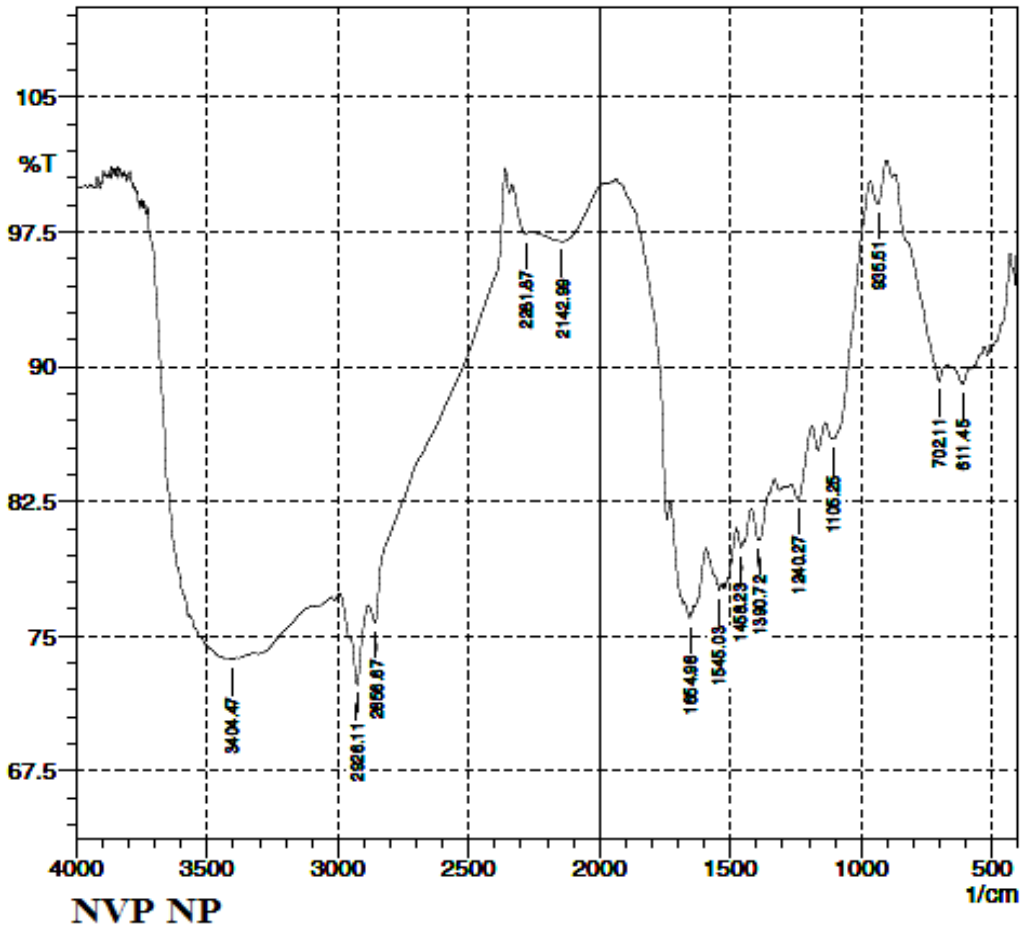


Fig 7 IR SPECTRUM OF NEVIRAPINE NANOPARTICLES

B. Differential scanning calorimetry (DSC)

In order to confirm the physical state of Nevirapine in the nanoparticles, DSC of the NVP, physical mixture of NVP and polymer, NVP nanoparticles and blank nanoparticles,was carried out and the results are shown in figures 8,9,10,11,12 and 13. The DSC thermogram of of NVP showed a sharp endothermic peak at 94.96°C, its melting point. The physical mixture of NVP and polymer, showed the same thermal behavior 93.04°C as the individual component, indicating that there was no interaction between the NVP and the polymer in the solid state. The DSC thermogram of blank nanoparticles showed a single endothermic peak at 80.72°C, the melting point of HSA. The nanoparticles of F1,F2 and F3, showed endothermic peak at 68.75°C, 81.59°C and 92.05°C respectively,which are the characterstic peaks of HSA shifted from 80.72°C , and there was no evidence for presence of the character peaks of NVP at 94.96°C in F1,F2 and F3. The change in thermogram indicate that NVP existed in an amorphrous form completely where as the HSA partially changes to amorphous state.

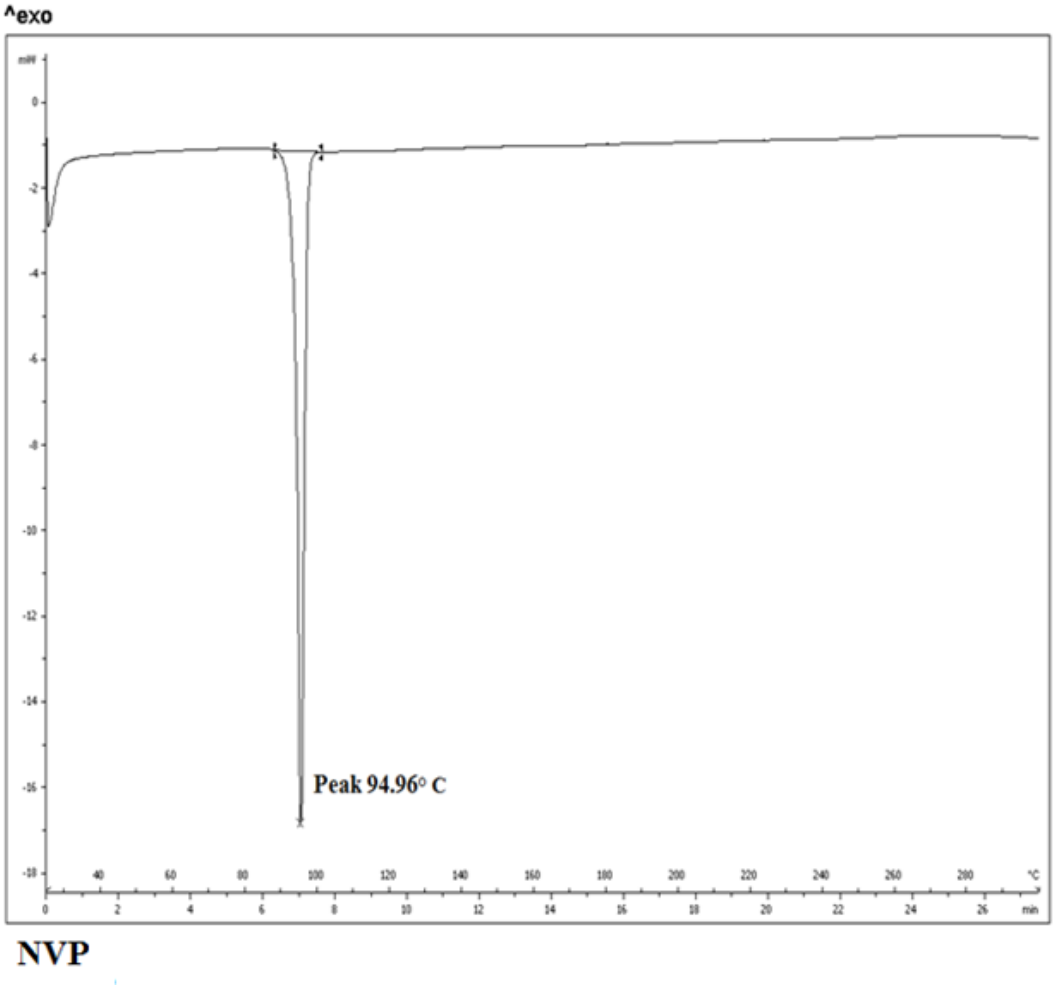


Fig 8 DSC thermogram of nevirapine

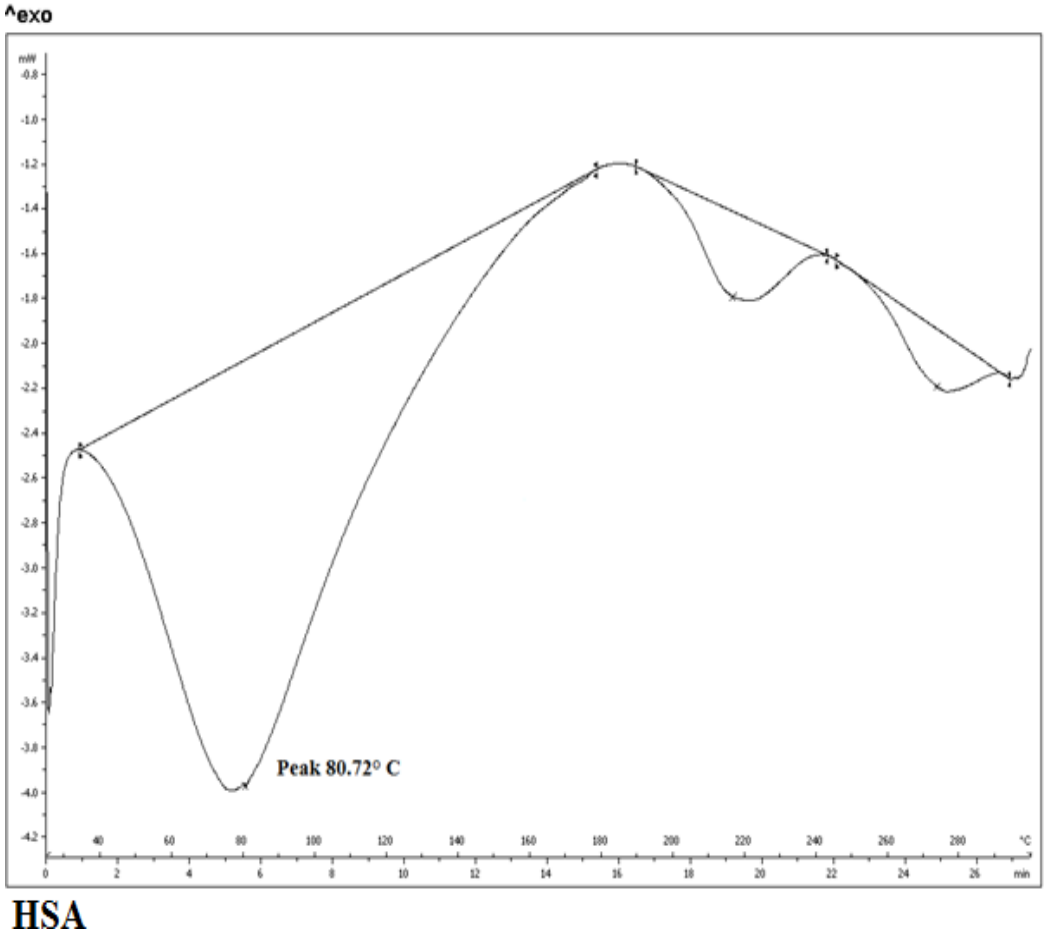


Fig 9 DSC thermogram of blank nanoparticles using Human serum albumin

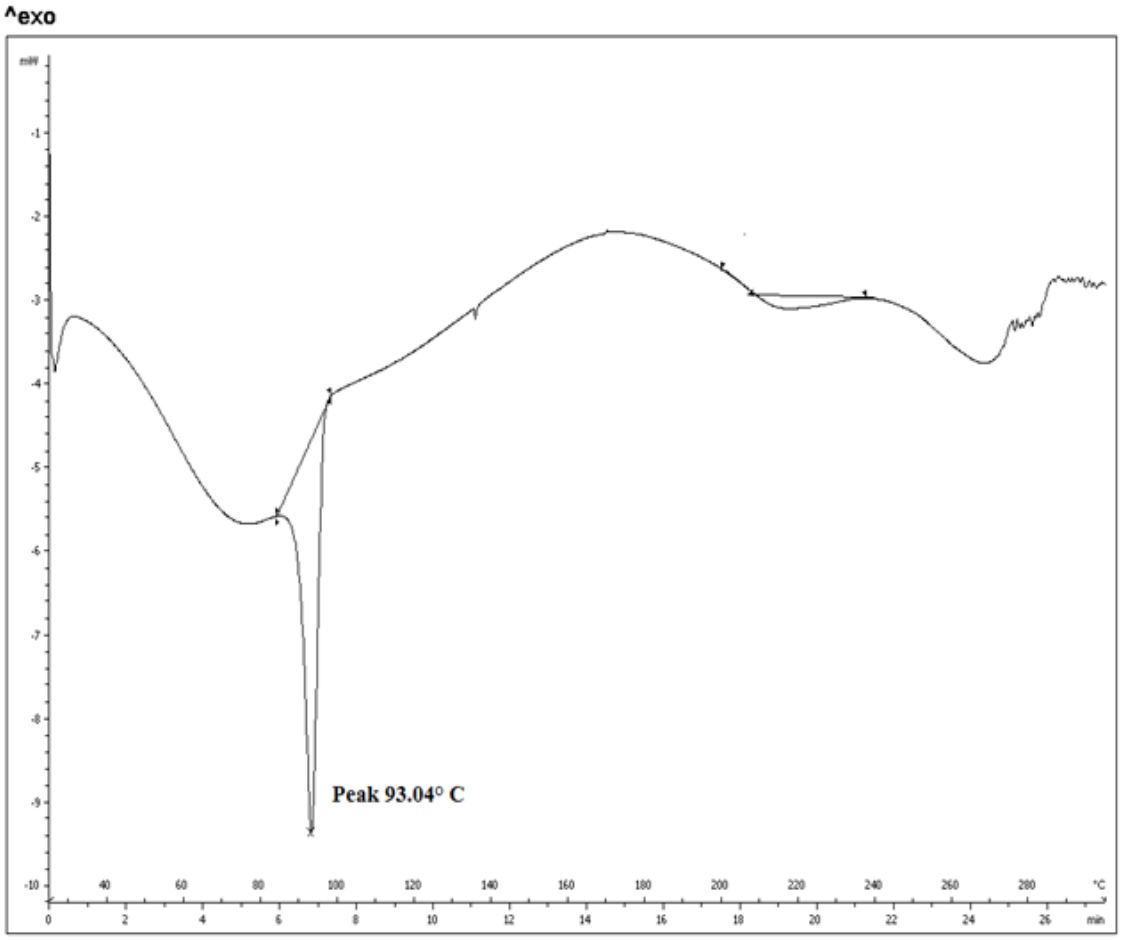


Fig 10 DSC thermogram of physical mixture of nevirapine and human serum albumin

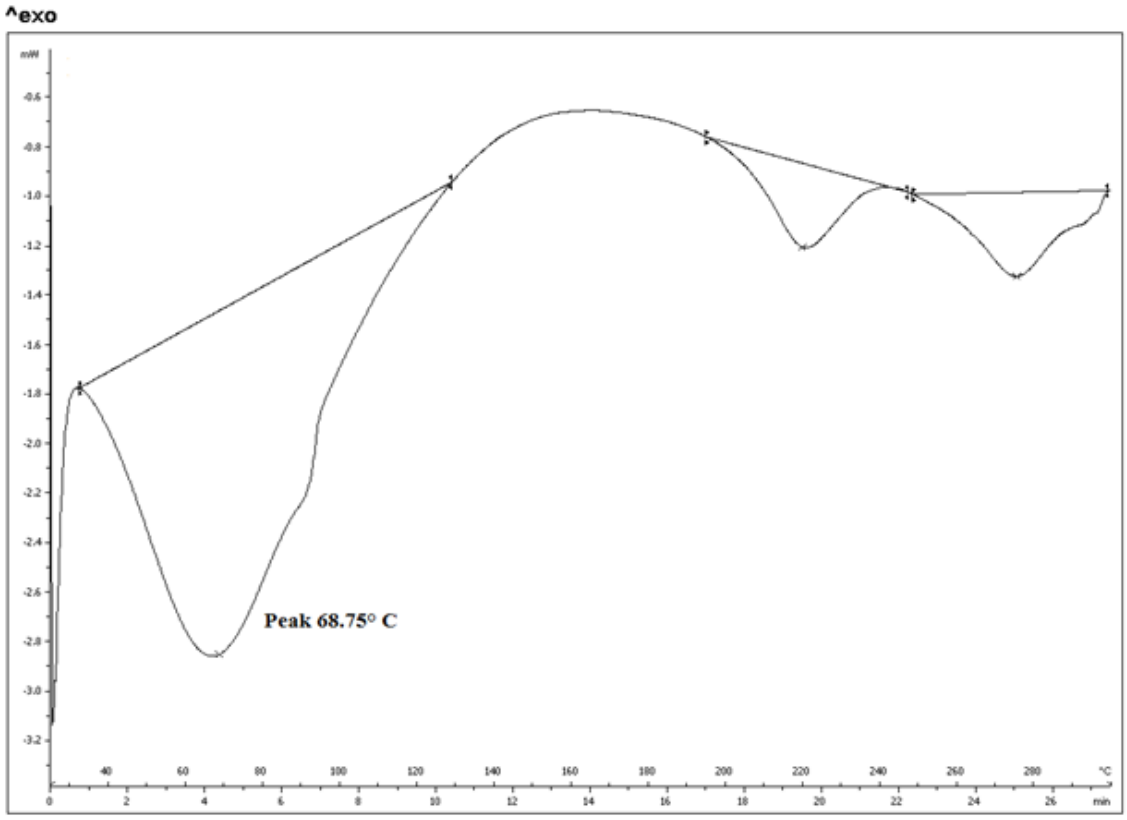


Fig 11 DSC thermogram of Nevirapine nanoparticles using Human serum albumin

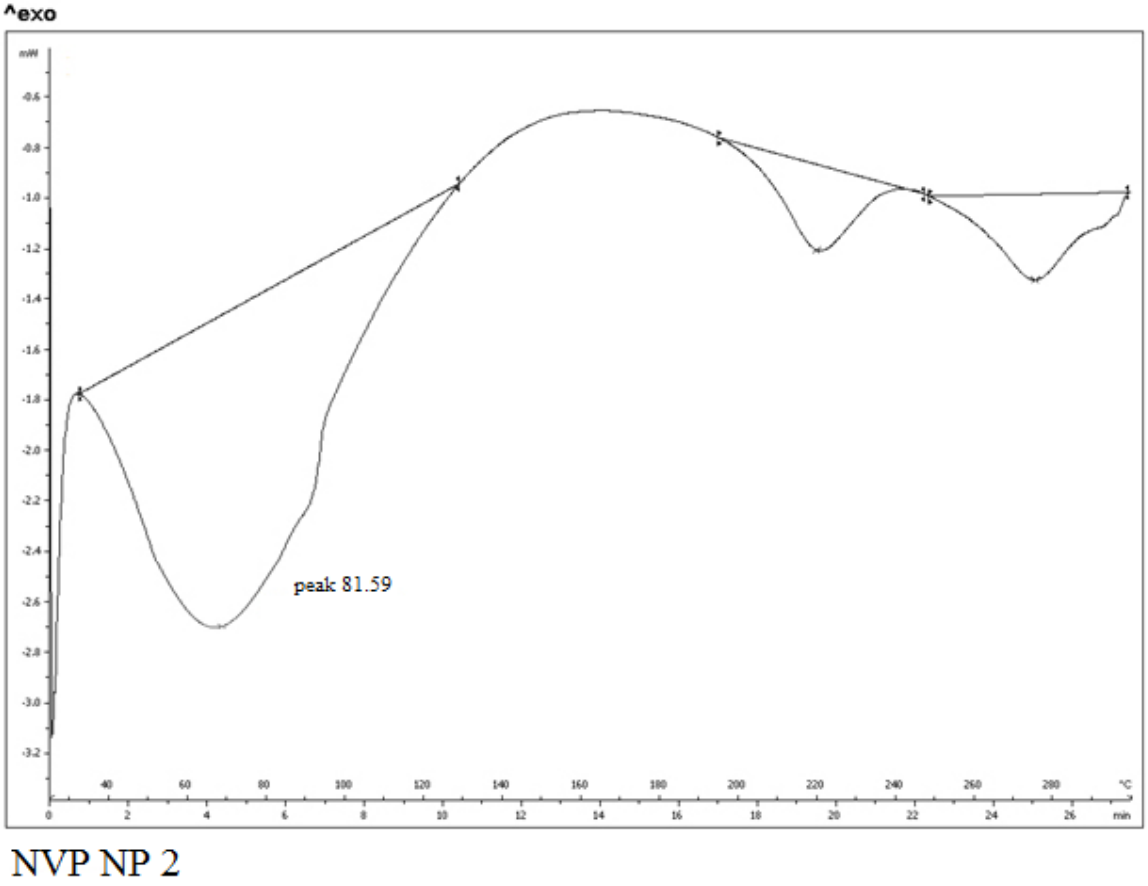


Fig 12 DSC thermogram of Nevirapine nanoparticles using Human serum albumin

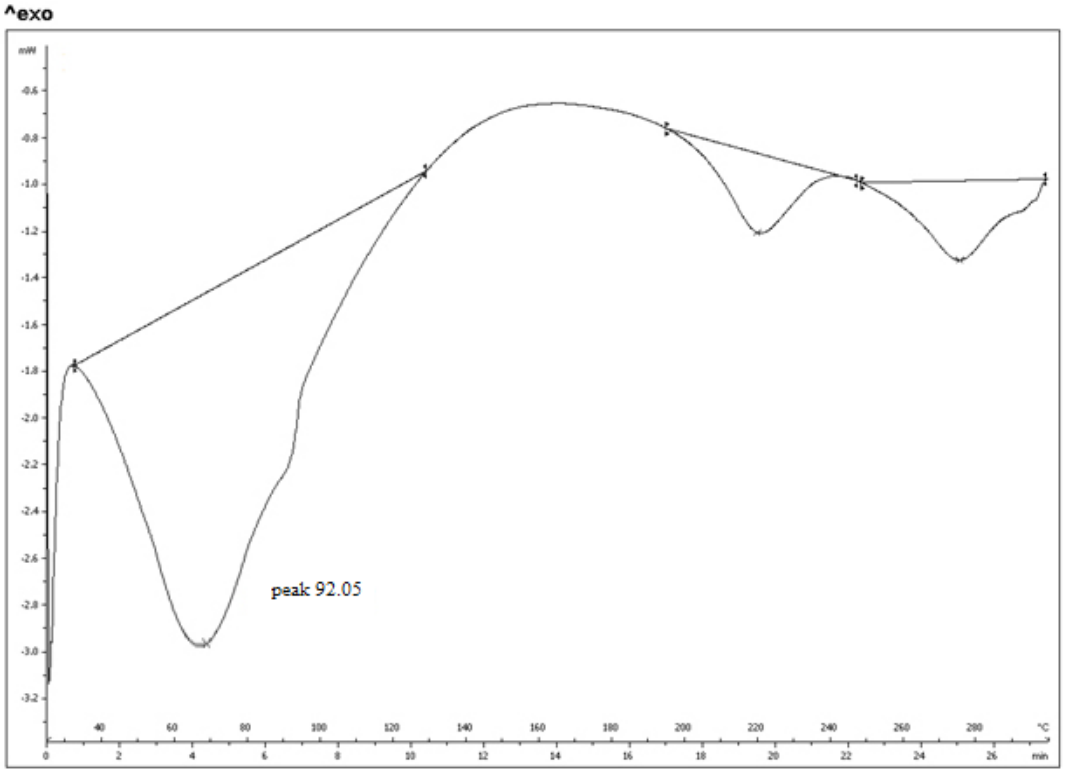


Fig 13 DSC thermogram of Nevirapine nanoparticles using Human serum albumin

9.2 Encapsulation efficiency and percentage yield:

The entrapment efficiency of F1,F2 and F3 were 58.54%, 68.44% and 73.12% respectively. Similarly the percentage yield increses in the concentration of HSA increases. The percentage yield of F1,F2 and F3 were 50.91,60.46 and 83.86 respectively. Table 12 shows the results of encapsulation efficiency and percentage yield of the nevirapine nanoparticles.

Formulation Code	Drug polymer ratio (mg)	Encapsulation efficiency (%)	Percentage yield (%)
F1	1:1	58.54	50.91
F2	1:2	68.44	66.46
F3	1:4	73.12	83.86

Table 12 encapsulation efficiency and percentage yield of the nanoparticles

Morphology:

The SEM of nanoparticles are shown in figures, The particles was formed disperse and irregular in shape at the drug:polymer ratio 1:1 in F1 and remained in the same characterstic at higher concentration of the polymer as seen in F2 and F3 formulations.

SEM IMAGES:

Fig 14 (F1)

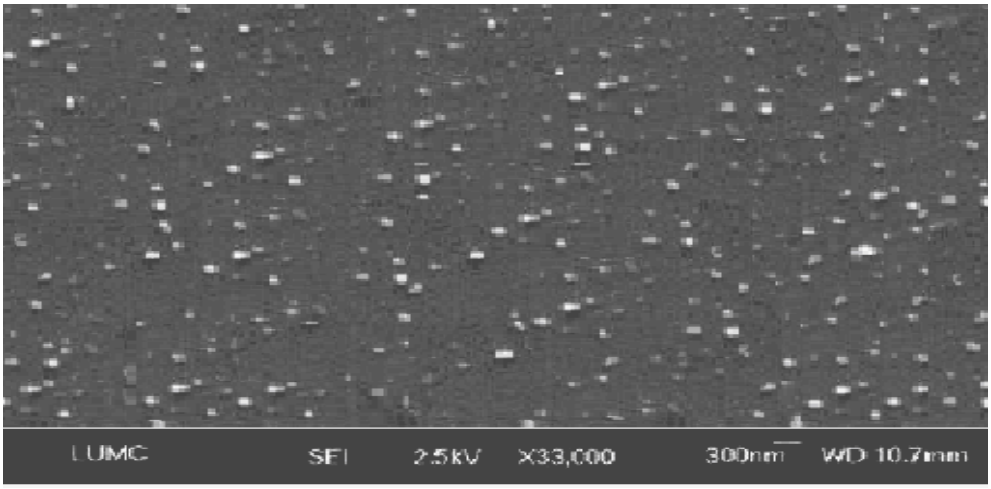


Fig 15 (F2)

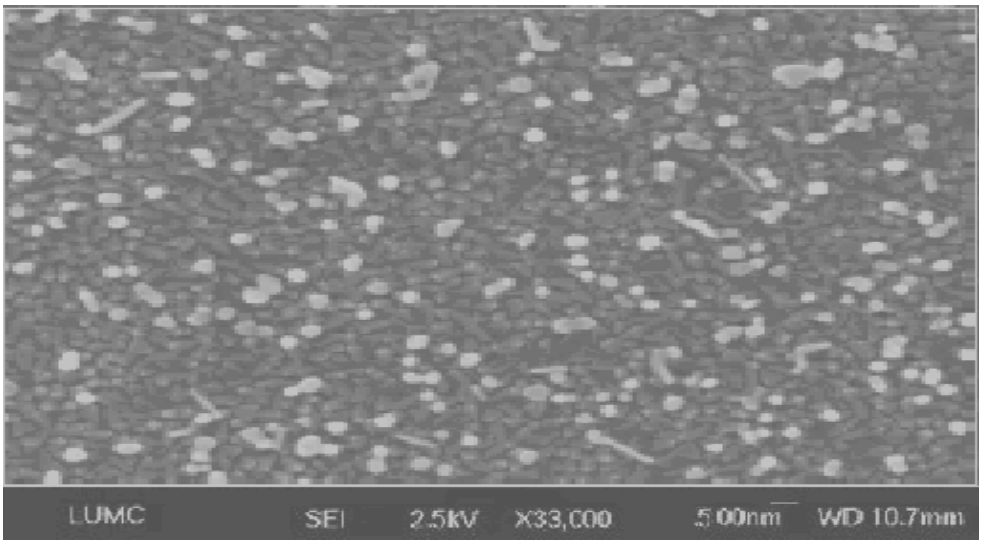
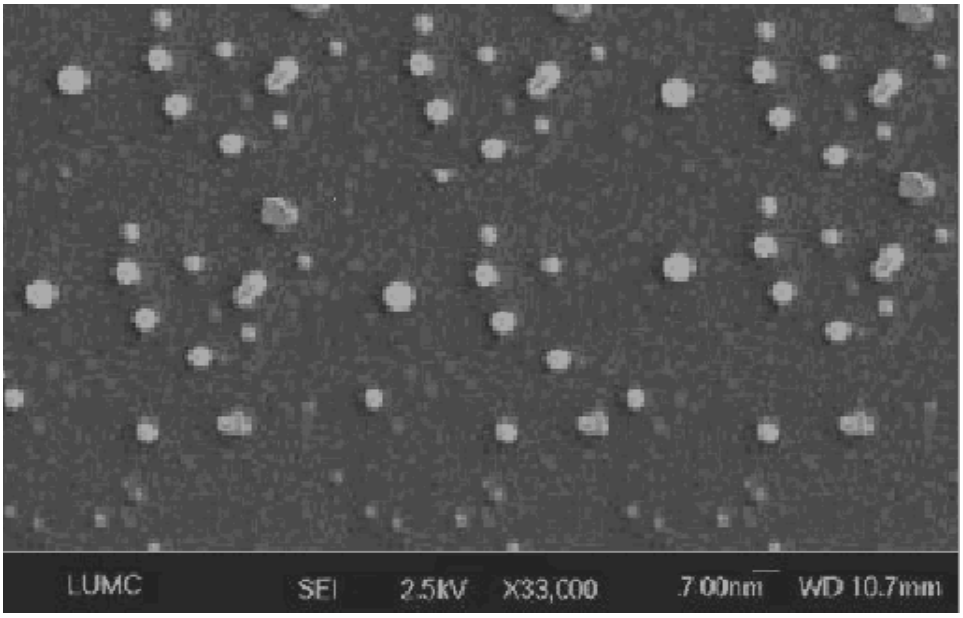


Fig 16 (F3)



9.3 PARTICAL SIZE:

The mean particle size of F1, F2 and F3 formulations , obtained by zetasizer analysis were 298,495 and 698nm (table 13) respectively. The particle size of nanoparticles was found increases with increase in the polymer concentration.

Table 13 Particle size, Zeta potential and PDI of nevirapine loaded nanoparticles

Formulation code	Drug polymer ratio (mg)	Particle size	Zeta potential(mv)	PDI
F1	1:1	298±0.25nm	-0.27±1.2mV	0.195±0.35
F2	1:2	495±0.12nm	-0.22±2.8mV	0.238±0.39
F3	1:4	698±0.52nm	-0.18±1.8mV	0.435±0.51

9.3.1 Zeta potential:

The zeta performed of F1, F2 and F3 formulations are shown in table 13. The zeta potential of nanoparticles ranges from -0.18 to -0.27 the least zeta potential with F3 and the ahighest value with F1. The zeta potential decreases with increase in the concentration of the polymer.

9.3.2 Polymer dispersive index:

The PDI of F1,F2 and F3 are shown in table. The PDI of all these formulations easily formed to be less than 0.5 indicating homogenous dispersion of the drug. The results are showed in table 13.

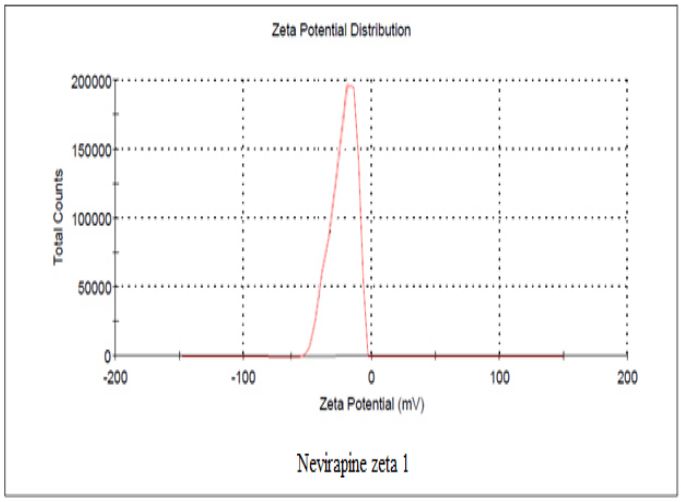


Fig 17 Nevirapine zeta

9.4 In vitro release of nevirapine nanoparticles

The dissolution profile of F1,F2 and F3 are presented in tables 14,16 and 18. The present drug dissolved at every time point interval significantly dispersed between F1,F2 and F3. The dissolution rate with F2 and F3 was slower compared to F1

The t_{50} values of F1, F2 and F3 were also compared. The data are F1 Vs F2 ($P<0.05$); F1 Vs F3 ($P<0.001$); F2 Vs F3 ($P<0.05$). There was no significant difference in t_{50} value between F1 and F2, however significant differences observed between F1 and F3.

Table 14 In vitro release data for nevirapine loaded nanoparticles formulation (F1)

Time (hrs)	Cumulative Percent Drug Release			
	Trial 1	**Trial 2**	**Trial 3**	**Mean ± SD**
0	0	0	0	0
0.5	16.65	16.45	16.53	16.51± 0.62
1	23.35	23.29	22.28	23.22± 0.49
2	29.68	29.54	29.61	29.58± 0.69
4	36.68	36.54	36.57	36.59± 0.68
‘6	43.59	43.42	43.45	43.48± 0.67
8	56.81	56.69	56.65	56.72.± 0.60
10	63.65	63.45	63.49	63.52± 0.67
12	70.59	70.39	70.45	70.48± 0.52
24	91.29	91.45	91.25	91.36± 0.68

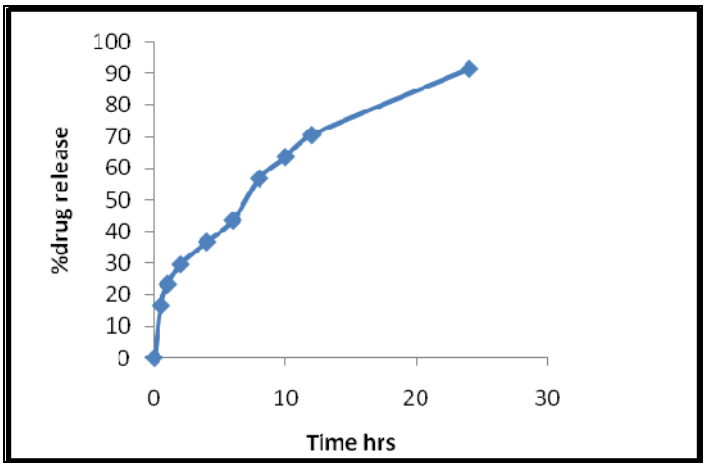


Fig 18 Percentage Drug Release

Table 15 Kinetic release data for nevirapine loaded nanoparticles(F1)

Time in hrs	Square root of time	Log time	Cumulative %drug release	Log cumulative %drug release	Cumulative %drug remaining	Log cumulative %drug remaining
0	0	0	0	0	100	2
0.5	0.707	-0.301	16.51	1.217	83.49	1.921
1	1	0	23.22	1.365	98.63	1.994
2	1.414	0.301	29.58	1.47	98.53	1.993
4	2	0.602	36.59	1.563	98.43	1.993
6	2.449	0.778	43.48	1.638	98.36	1.992
8	2.828	0.903	56.72	1.753	98.24	1.992
10	3.162	0.499	63.52	1.802	98.19	1.992
12	3.464	0.539	70.48	1.848	98.15	1.991
24	4.898	1.38	91.36	1.96	98.04	1.991

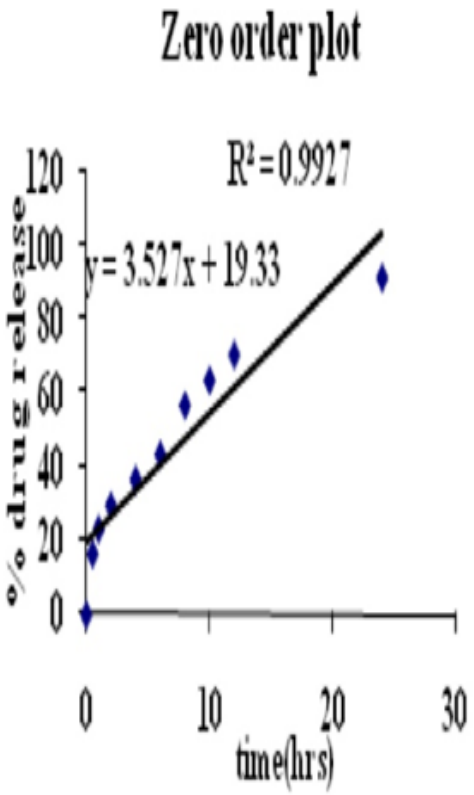


Fig 18 (a)

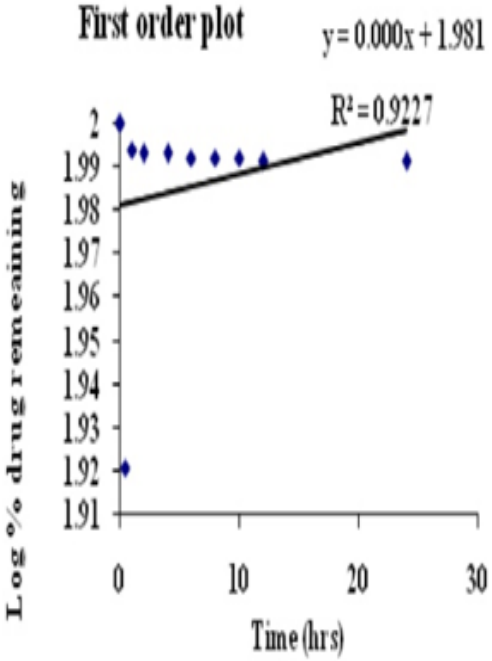


Fig 18 (b)

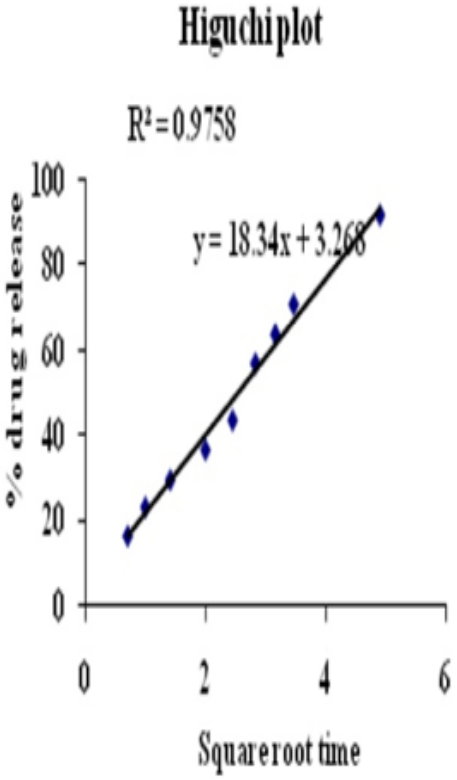


Fig 18 (c)

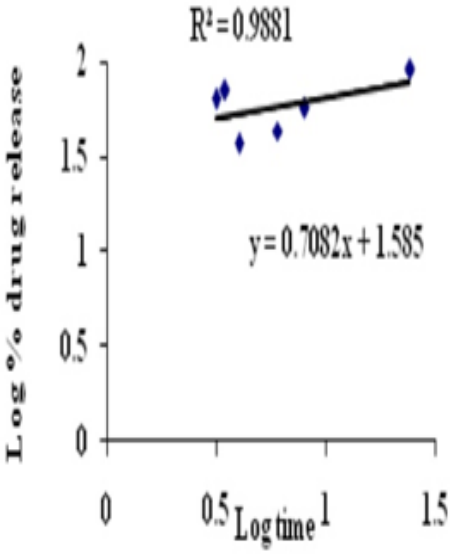


Fig 18 (d)

Table 16 In vitro release data for nevirapine loaded nanoparticles formulation (F2)

Time (hrs)	Cumulative Percent Drug Release			
	Trial 1	**Trial 2**	**Trial 3**	**Mean ± SD**
0	0	0	0	0
0.5	15.52	15.39	15.35	15.45± 0.57
1	21.65	21.35	21.52	21.47± 0.67
2	27.62	27.53	27.49	27.56± 0.61
4	33.75	33.59	33.52	33.66± 0.58
6	40.51	40.39	40.35	40.42± 0.61
8	46.65	46.56	46.49	46.53.± 0.59
10	59.49	59.32	59.35	59.38± 0.47
12	65.52	65.45	65.42	65.42± 0.55
24	83.62	83.45	83.49	83.51± 0.52

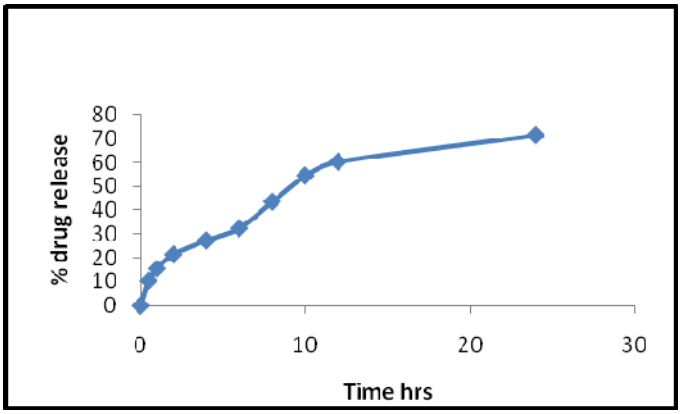


Fig 18 Percentage of Drug Release

Table 17 Kinetic release data for nevirapine loaded nanoparticles(F2)

Time in hrs	Square root of time	Log time	Cumulative %drug release	Log cumulative %drug release	Cumulative %drug remaining	Log cumulative %drug remaining
0	0	0	0	0	100	2
0.5	0.707	0.301	15.45	1.118	98.88	1.995
1	1.414	0	21.47	1.331	98.66	1.994
2	1.732	0.301	27.56	1.44	98.56	1.993
4	2	0.602	33.66	1.527	98.47	1.993
6	2.449	0.778	40.42	1.606	83.94	1.923
8	2.828	0.903	46.53	1.667	98.33	1.992
10	3.162	1	49.38	1.693	98.3	1.992
12	3.464	1.079	65.48	1.816	98.18	1.992
24	4.898	1.38	83.51	1.921	98.07	1.991

Fig 19 (a)

F

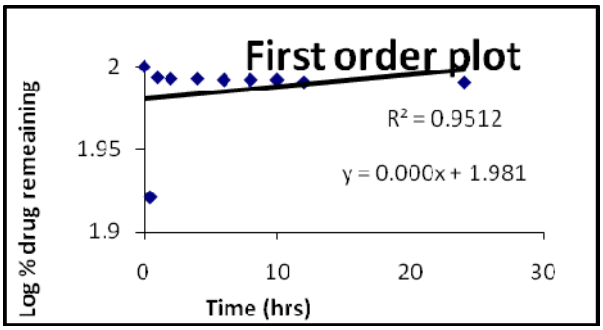


Fig 19 (b)

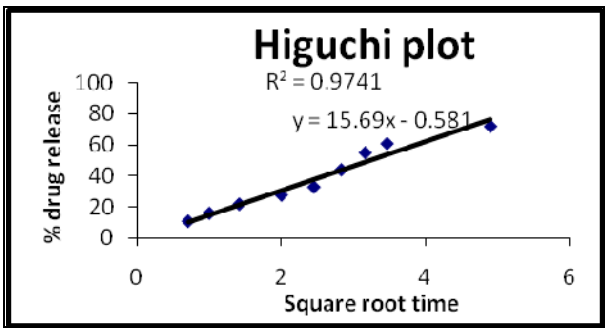


Fig 19 (c)

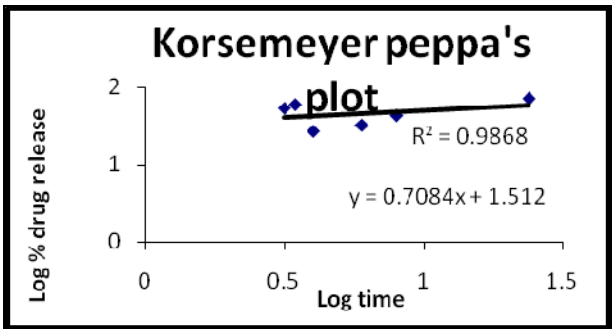


Fig 19 (d)

Table 18 In vitro release data for nevirapine loaded nanoparticles formulation (F3)

Time (hrs)	Cumulative Percent Drug Release			
	Trial 1	Trial 2	Trial 3	Mean ± SD
0	0	0	0	0
0.5	10.65	10.49	10.45	10.52± 0.64
1	15.75	15.59	15.55	15.63± 0.57
2	21.62	21.45	21.42	21.50± 0.60
4	27.42	27.32	27.29	27.37± 0.62
6	32.65	32.49	32.52	32.56± 0.59
8	43.75	43.59	43.62	43.68.± 0.56
10	54.75	54.65	54.69	54.62± 0.60
12	60.61	60.52	60.45	60.49± 0.56
24	71.68	71.51	71.46	71.55± 0.64

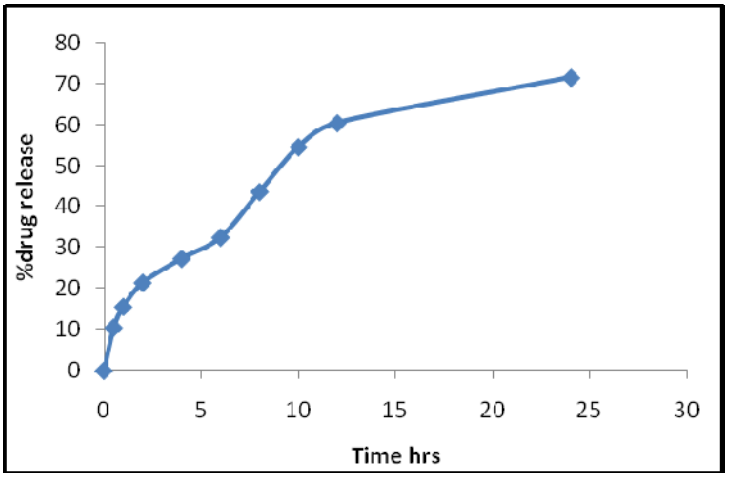


Fig 20 Percentage of Drug Release

Table 19 Kinetic release data for nevirapine loaded nanoparticles(F3)

Time	sqrt	log time	% drug rel	log % drug release	% drug remaining	log % drug remaining
0	0	0	0	0	100	2
0.5	0.707	-0.301	10.65	1.027	98.97	1.995
1	1	0	15.75	1.197	98.8	1.994
2	1.414	0.301	21.62	1.334	98.66	1.994
4	2	0.602	27.42	1.438	98.56	1.993
6	2.449	0.778	32.65	1.513	98.56	1.993
8	2.828	0.903	43.75	1.64	98.36	1.992
10	3.162	0.499	54.75	1.738	98.26	1.992
12	3.464	0.539	60.61	1.782	98.21	1.992
24	4.898	1.38	71.68	1.855	98.14	1.991

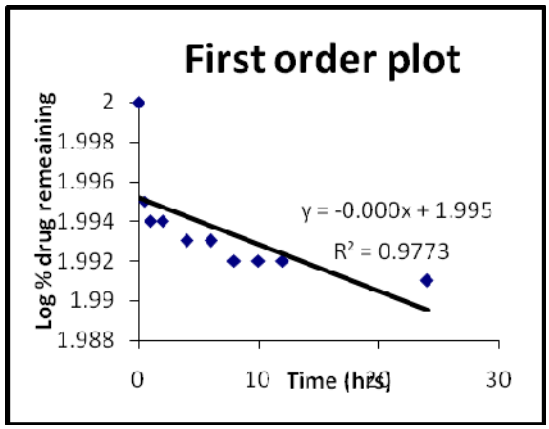


Fig 21 (a)

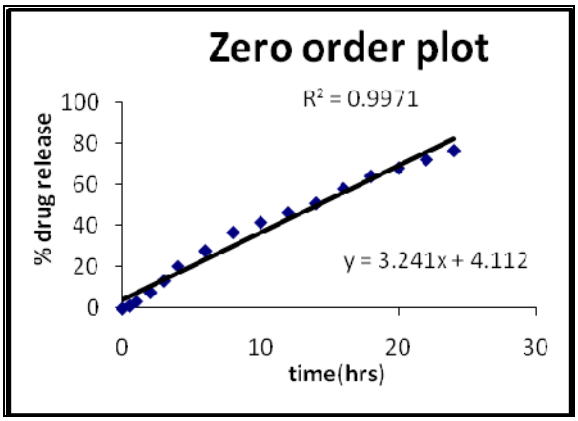


Fig 21 (b)

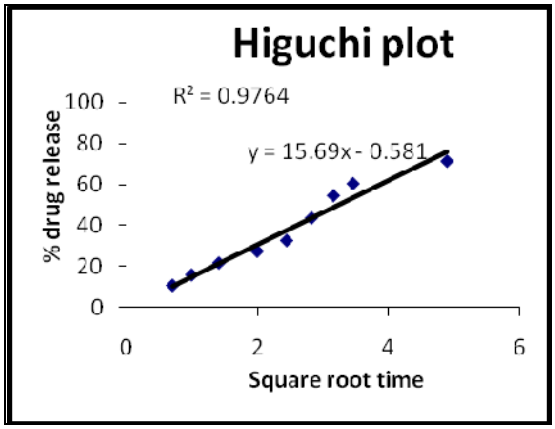


Fig 21 (c)

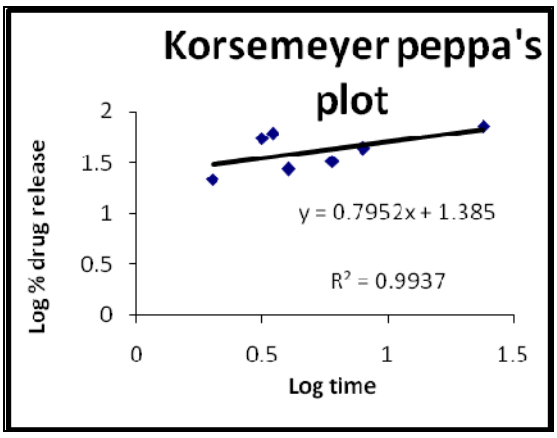


Fig 21 (d)

Table 20 Comparative study for dissolution of nevirapine loaded nanoparticles

Time (hrs)	Cumulative Percent Drug Release		
	F1	F2	F3
0	0	0	0
0.5	16.51± 0.62	15.45± 0.57	10.52± 0.64
1	23.22± 0.49	21.47± 0.67	15.63± 0.57
2	29.58± 0.69	27.56± 0.61	21.50± 0.60
4	36.59± 0.68	33.66± 0.58	27.37± 0.62
6	43.48± 0.67	40.42± 0.61	32.56± 0.59
8	56.72.± 0.60	46.53.± 0.59	43.68.± 0.56
10	63.52± 0.67	59.38± 0.47	54.62± 0.60
12	70.48± 0.52	65.42± 0.55	60.49± 0.56
24	91.36± 0.68	83.51± 0.52	71.55± 0.64

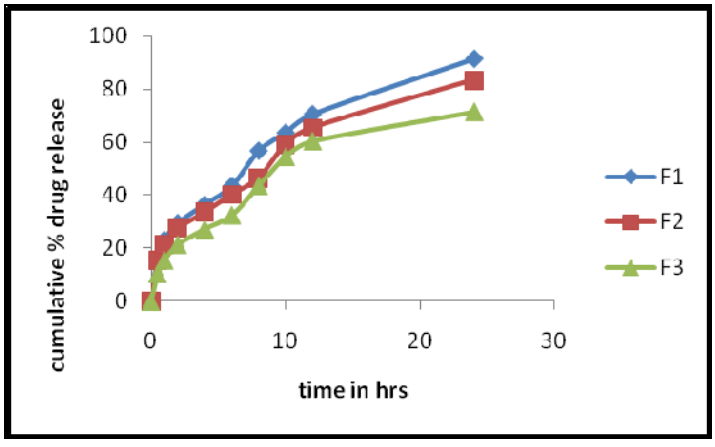


Fig 22 Comparative Dissolution Profile

Table 21 In vitro release kinetic data for nevirapine loaded nanoparticles

Formulation	Zero order	First order	Higuchi Matrix	Peppas plot	
				r^2 value	'n' value
1:1	0.9927	0.9227	0.9758	0.9881	0.7082
1:2	0.9930	0.9512	0.9741	0.9868	0.7084
1:4	0.9971	0.9773	0.9764	0.9937	0.7952

K_0- Zero order rates constant

K_1 - First order rate constant

R – Coefficient of correlation

N – Diffusion exponent

The regression co-efficient values are zero order plots wew compared. It was observed that the "R" value of zero order plit were in the range of 0.9927 to 0.9971 from all the three formulations. The "R" values of first order plot were in the range of 0.9925 to 0.9773, based on the highest regression values (R), the best fit model for F1, F2 and F3. Followed zero order release. The "R" value of the linear regression for korsmeyer plot were found in the range of 0.9881 to 0.9937 for all the formulation indicating that the data fit into the korsmeyer plot model well, and the drug release was found to be predominantely controlled by swelling process.

When the slope n values of formulation F1,F2 and F3 in korsmeyer equation (F1=0.7082, F2=0.7084 and F3=0.7952) were compared, it was observed that increasing polymer concentration in the nanoparticle led to increase in the slope value from 0.7082 to 0.7952 indicating that the drug release by case II transport mechanism. (n>1) class II transport mechanism which involves swelling controlled release of the drug.

Turkey – Kramer multiple camparision test : t_{90}

The release data were subject to ANOVA with Turkey- Kramer comparision test F2 showed release pattern as compared F1 ($p<0.05$) .Similar release behavior was observed between F3 and F1 ($p<0.001$) and between F3 and F2 ($p<0.05$).The result suggest that the

slow drug release characterstics appear to be influenced by polymer concentration. Table 22, 23.

Table 22 ANALYSIS OF VARIANTS

Formulation code	F1	F2	F3
Mean	43.146	38.346	33.888
Standard deviation	27.621	24.465	23.300
sample size (N)	10	10	10
std error of mean	8.735	7.736	7.368
Lower 95% conf. limit	62.904	55.846	50.555
upper 95% conf. limit	56.41	52.8	48.48
Minimum	0	0	0
Median	40.035	37.040	30.035
Maximum	91.360	83.510	71.680
Normality test KS	0.09518	0.1260	0.1212
Normality test P value	>0.10	>0.10	>0.10
Passed normality test	yes	yes	yes

Table 23 Turkey –kramer multiple comparison test:t_{90}

Comparision	Mean difference	"q" value	"p" value
F1 Vs F2	4.800	4.532	P< 0.05
F1 Vs F3	9.258	8.742	P< 0.001
F2 Vs F3	4.458	4.209	P< 0.05

The **"**P" value is <0.0001, considered extremely significant. Variation among column means is significantly greater than expected by chance.

If the value q is greater than 3.609 then the p value is less than 0.05.

9.4.2 DIFFUSION STUDIES:

Cumulative percentage drug released for F1, F2 and F3 at 24 hrs was 92.40%, 83.43% and 76.23% (Table 24,26 and 28) respectively. The invitro release profile of all the formulation showed a slow and steady release pattern approximately in zero order release. There was no burst release observed in all the formulations indicating absence of drug load along the surface of the nanoparticles.

The release data were subjected to ANOVA with Tukey –Kramer multiple comparison test. F3 showed slower release pattern as compared to F2 ($p<0.001$). Similar release behavior was observed between F2 and F1 ($p<0.001$), and between F3 and F1 ($p< 0.001$). The results suggest that the slow drug release characteristic appears to be influenced by polymer molecular weight.

The t_{50} values of F1, F2 and F3 were also compared. The data are F1 Vs F2 ($P>0.05$); F1 Vs F3 ($P<0.001$); F2 Vs F3 ($P<0.01$). There was no significant difference in t_{50} value between F1 and F2, however significant differences observed between F2 and F3.

Table 24 In vitro release data for nevirapine nanoparticles (F1)

Time (hrs)	Cumulative Percent Drug Release			
	Trial 1	**Trial 2**	**Trial 3**	**Mean ± SD**
0	0	0	0	0
0.5	1.735	1.731	1.738	1.739± 0.0035
1	4.932	4.921	4.925	4.922± 0.0055
2	9.786	9.685	9.689	9.686± 0.0571
3	15.489	15.475	15.484	15.485± 0.0070
4	25.549	25.531	25.531	25.535± 0.0110
6	30.189	30.179	30.179	30.182.± 0.0682
8	37.398	37.398	37.392	37.394± 0.0030
10	43.141	43.132	43.132	43.129± 0.0062
12	49.998	49.986	49.986	49.996± 0.0061
14	52.238	52.241	52.241	52.238± 0.0035
16	58.490	58.481	58.481	58.491± 0.0045
18	68.835	68.821	68.821	68.825± 0.0073
20	77.579	77.569	77.565	77.572± 0.0072
22	84.792	84.785	84.784	84.789± 0.0007
24	92.412	92.401	92.402	92.407± 0.0060

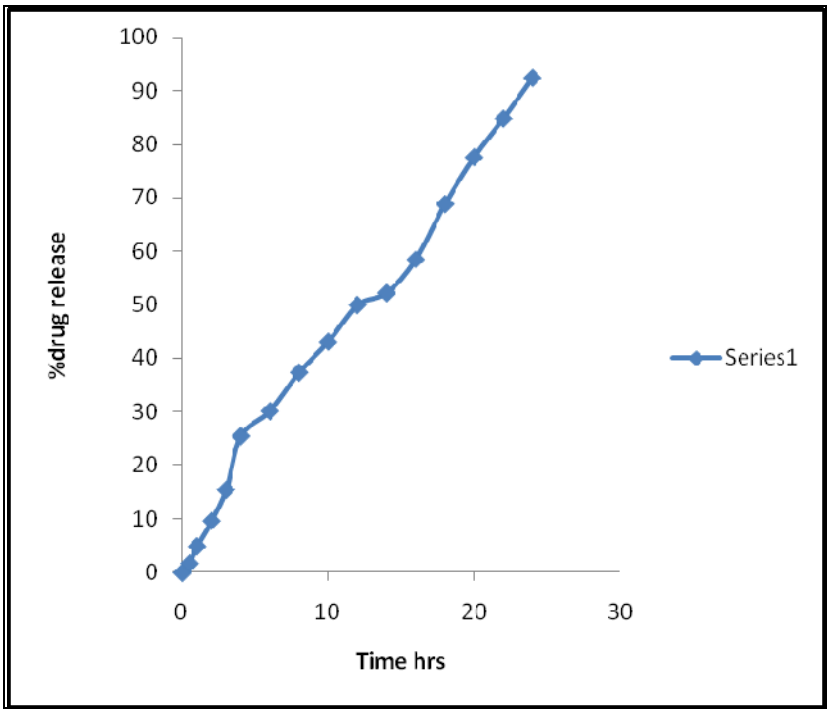


Fig 23 Percentage Drug Release

Table 25 Kinetic release data for nevirapine nanoparticles (F1)

ime in hrs	Square root of time	Log time	Cumulative %drug release	Log cumulative %drug release	Cumulative %drug remaining	Log cumulative %drug remaining
0	0	0	0	0	100	2
0.5	0.707	-0.301	1.73	0.238	99.76	1.998
1	1	0	4.92	0.691	99.3	1.996
2	1.414	0.301	9.68	0.985	99.01	1.995
3	1.732	0.477	15.48	1.189	98.81	1.994
4	2	0.602	25.53	1.407	98.59	1.993
6	2.449	0.778	30.17	1.479	98.52	1.993
8	2.828	0.903	37.39	1.572	98.42	1.993
10	3.162	1	43.12	1.634	98.36	1.992
12	3.464	1.079	49.98	1.698	98.3	1.992
14	3.741	1.146	52.24	1.718	98.28	1.992
16	4	1.204	58.48	1.767	98.23	1.992
18	4.242	1.255	68.82	1.837	98.16	1.991
20	4.472	1.301	77.56	1.889	98.11	1.991
22	4.69	1.342	84.78	1.928	98.07	1.991
24	4.898	1.38	92.4	1.965	98.03	1.991

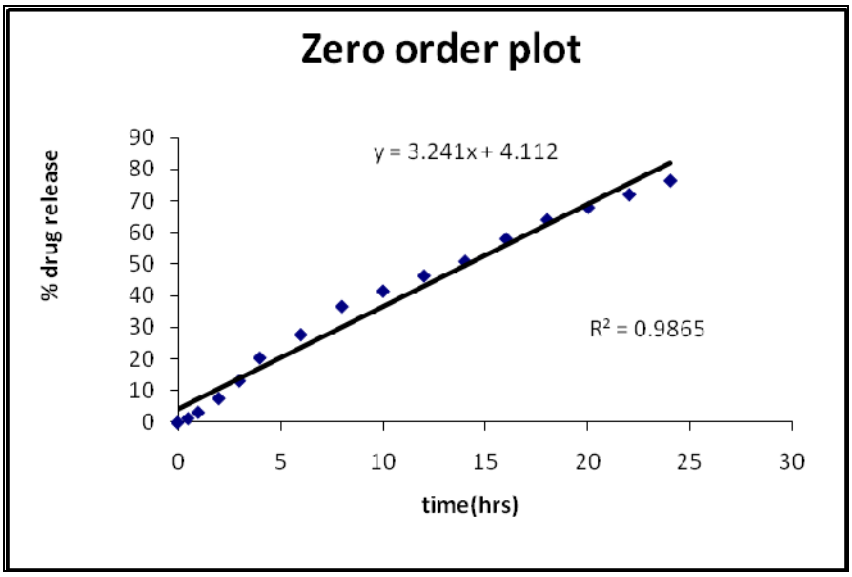


Fig 24 (a)

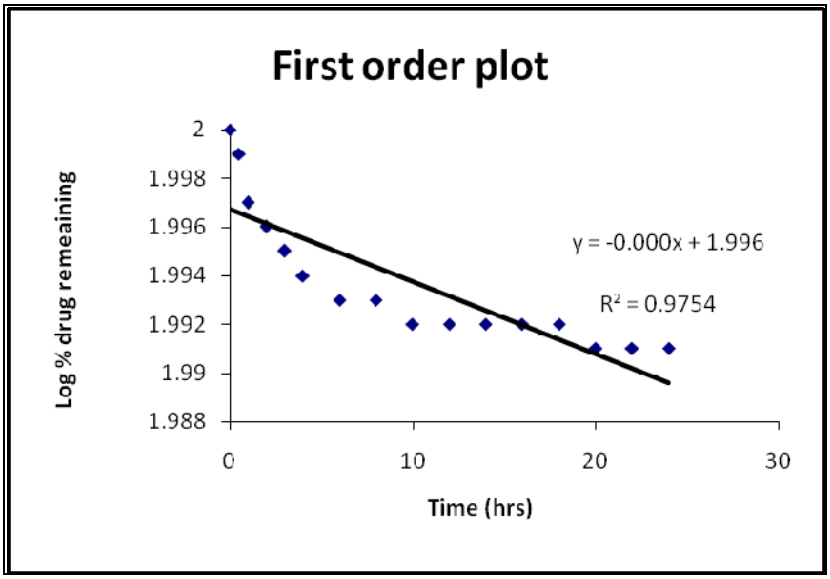


Fig 24 (b)

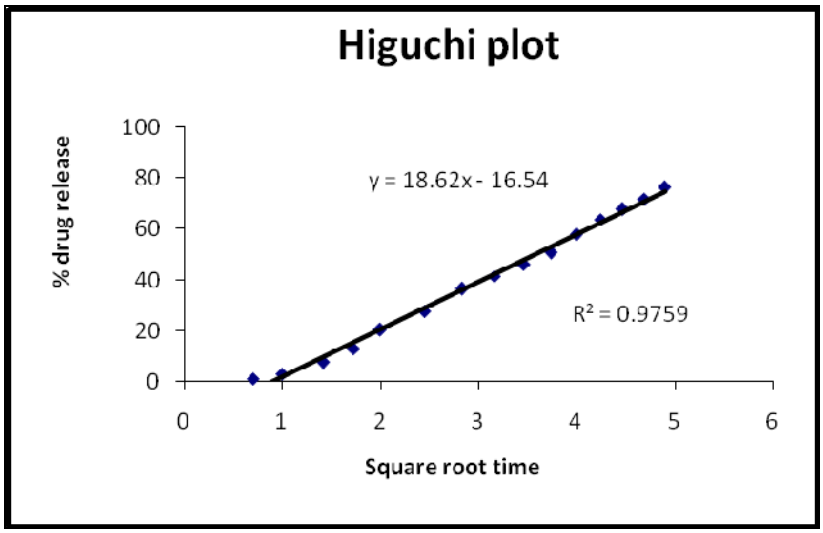


Fig 24 (c)

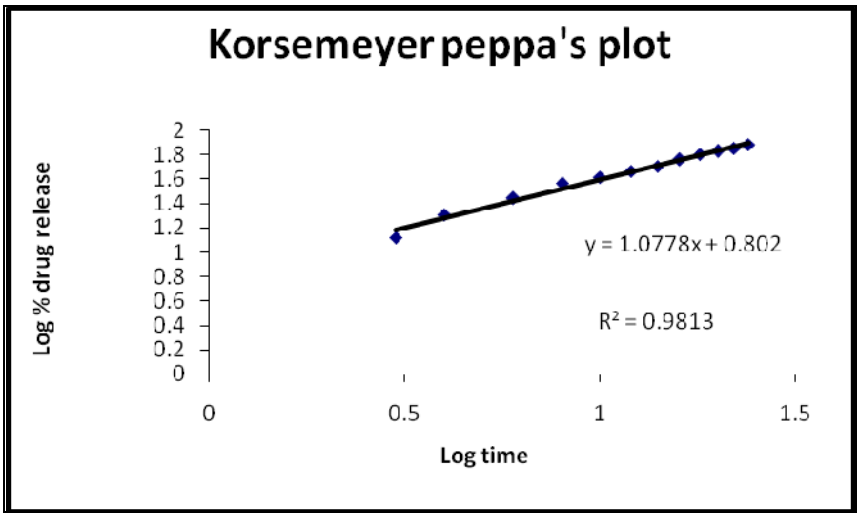


Fig 24 (d)

Table 26 In vitro release data for nevirapine nanoparticles(F2)

Time (hrs)	Cumulative Percent Drug Release			
	Trial 1	**Trial 2**	**Trial 3**	**Mean ± SD**
0	0	0	0	0
0.5	1.381	1.372	1.374	1.376± 0.0047
1	3.512	3.492	3.494	3.500± 0.0110
2	8.782	8.772	8.776	8.777± 0.0050
3	13.959	13.929	13.925	13.932± 0.0185
4	22.841	22.819	22.816	22.823± 0.194
6	29.551	29.521	29.525	29.536± 0.016
8	36.998	36.996	36.999	36.997± 0.0015
10	42.865	42.853	42.858	42.859± 0.0060
12	48.441	48.432	48.429	48.437± 0.0062
14	51.102	51.106	51.114	51.110± 0.583
16	57.815	57.809	57.804	57.810± 0.0055
18	62.401	62.381	62.382	67.383± 0.011
20	69.639	69.629	69.622	69.360± 0.0085
22	75.784	75.759	75.752	75.762± 0.0168
24	83.441	83.429	83.424	83.432± 0.0083

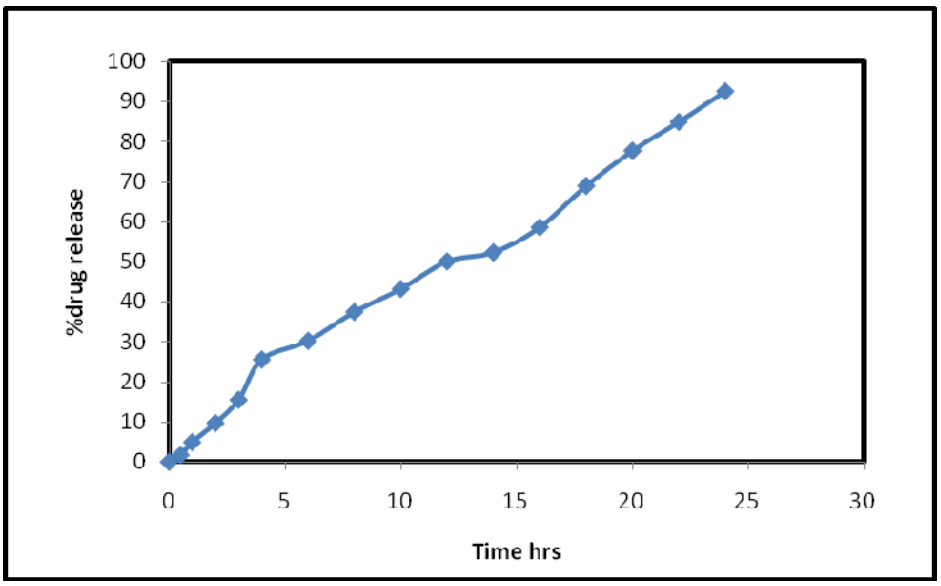


Fig 25 Percentage of Drug Release

Table 27 Kinetic release data for nevirapine nanoparticles (F2)

Time in hrs	Square root of time	Log time	Cumulative %drug release	Log cumulative %drug release	Cumulative %drug remaining	Log cumulative %drug remaining
0	0	0	0	0	100	2
0.5	0.707	-0.301	1.37	0.136	99.86	1.999
1	1	0	3.5	0.544	99.45	1.997
2	1.414	0.301	8.77	0.942	99.05	1.995
3	1.732	0.477	13.92	1.143	98.85	1.994
4	2	0.602	22.81	1.358	98.64	1.994
6	2.449	0.778	29.52	1.47	9853	1.993
8	2.828	0.903	36.99	1.568	98.43	1.993
10	3.162	1	42.85	1.631	98.36	1.992
12	3.464	1.079	48.42	1.685	98.31	1.992
14	3.741	1.146	51.11	1.708	98.29	1.992
16	4	1.204	57.81	1.762	98.23	1.992
18	4.242	1.255	62.38	1.795	98.2	1.992
20	4.472	1.301	69.62	1.842	98.15	1.991
22	4.69	1.342	75.75	1.879	98.12	1.991
24	4.898	1.38	83.42	1.921	98.07	1.991

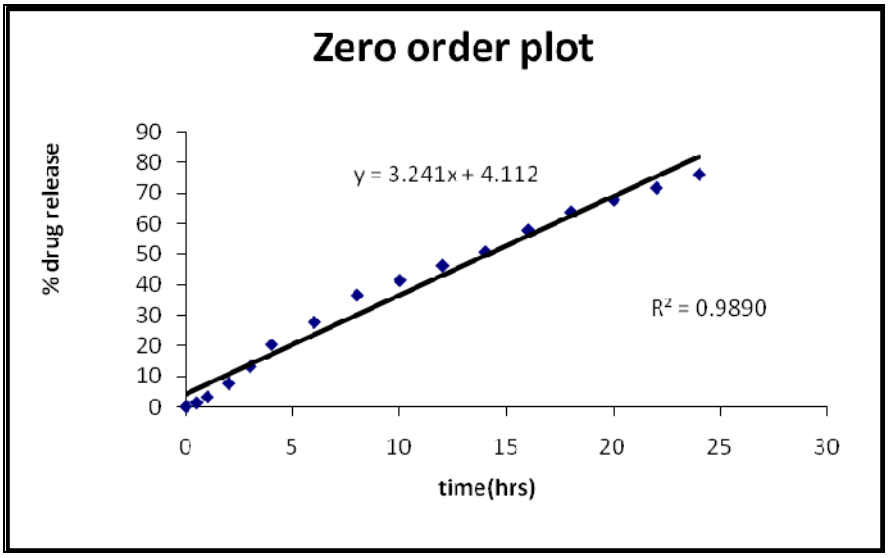


Fig 26 (a)

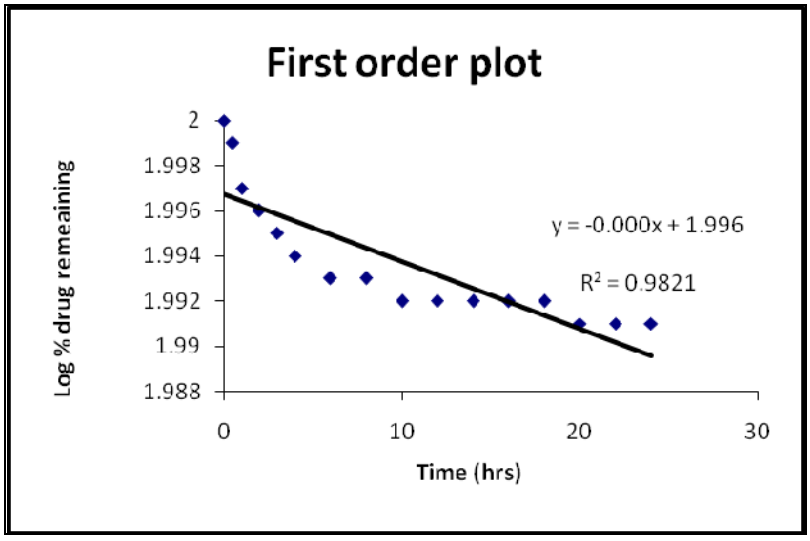


Fig 26 (b)

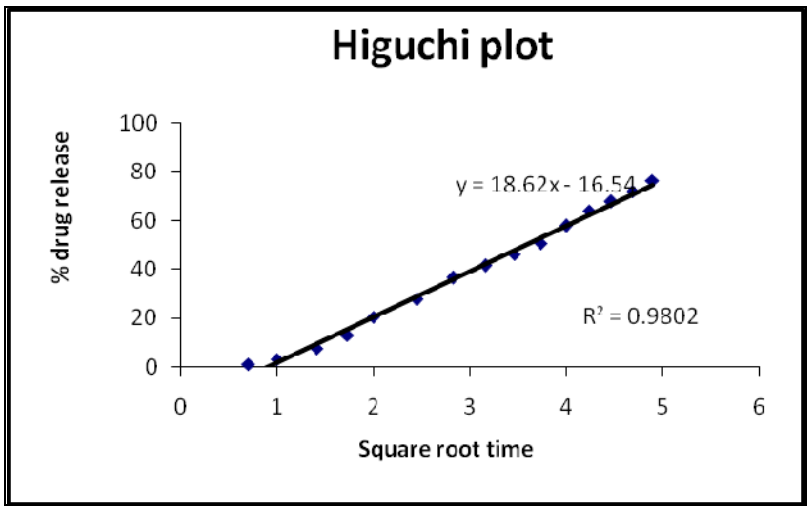


Fig 26 (c)

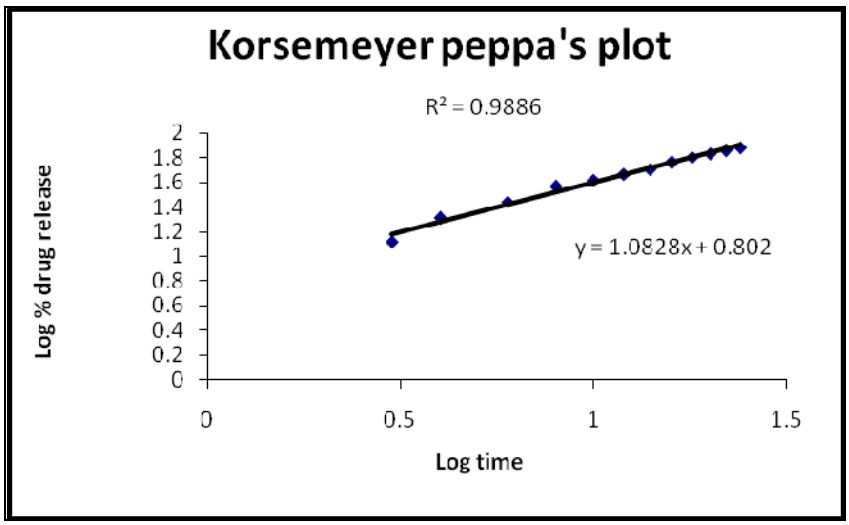


Fig 26 (d)

Table 28 In vitro release data for nevirapine nanoparticles (F3)

Time (hrs)	Cumulative Percent Drug Release			
	Trial 1	**Trial 2**	**Trial 3**	**Mean ± SD**
0	0	0	0	0
0.5	1.311	1.299	1.298	1.304± 0.0072
1	3.252	3.239	3.235	3.241± 0.0088
2	7.628	7.612	7.609	7.618± 0.0102
3	13.162	13.153	13.149	13.157± 0066
4	20.389	20.371	20.368	20.375± 0.0113
6	27.735	27.721	27.719	27.729± 0.0087
8	36.621	36.594	36.598	36.604± 0.0145
10	41.441	41.429	41.432	41.435± 0.0062
12	46.252	46.238	46.232	46.240± 0.0102
14	50.795	50.785	50.781	50.790± 0.0072
16	57.935	57.919	57.912	57.925± 0.0117
18	63.875	63.859	63.56	63.864± 0.0102
20	67.829	67.814	67.809	67.817± 0.0104
22	71.856	71.839	71.841	71.840± 0.0087
24	76.245	76.231	76.229	76.239± 0.0092

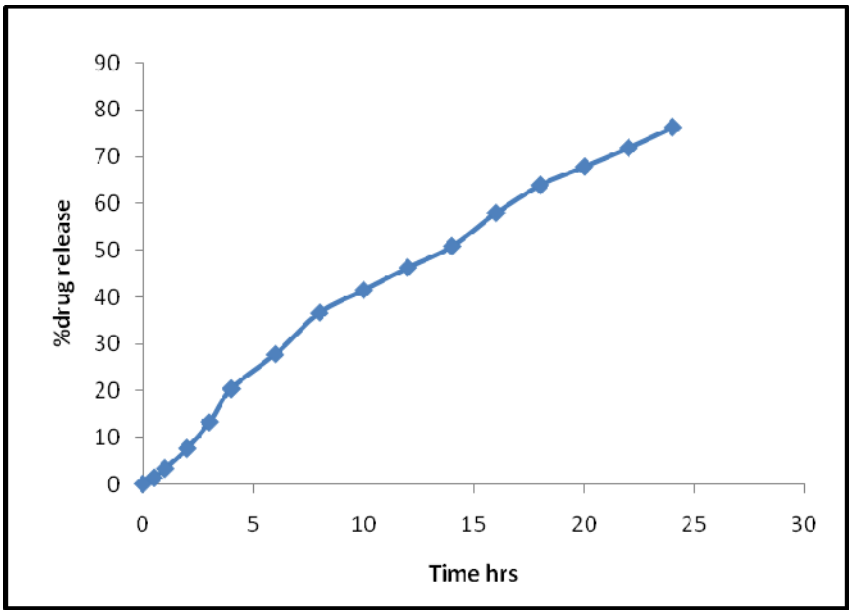


Fig 27 Percentage of Drug Release

Table 29 Kinetic release data for nevirapine nanoparticles (F3)

Time in hrs	Square root of time	Log time	Cumulative %drug release	Log cumulative %drug release	Cumulative %drug remaining	Log cumulative %drug remaining
0	0	0	0	0	100	2
0.5	0.707	-0.301	1.3	0.123	99.87	1.994
1	1	0	3.24	0.51	99.49	1.997
2	1.414	0.301	7.61	0.881	99.11	1.996
3	1.732	0.477	13.15	1.118	98.88	1.995
4	2	0.602	20.37	1.308	98.69	1.994
6	2.449	0.778	27.72	1.442	98.55	1.993
8	2.828	0.903	36.6	1.563	98.43	1.993
10	3.162	1	41.43	1.617	98.38	1.992
12	3.464	1.079	46.24	1.665	98.33	1.992
14	3.741	1.146	50.79	1.705	98.29	1.992
16	4	1.204	57.92	1.762	98.23	1.992
18	4.242	1.255	63.86	1.805	98.19	1.992
20	4.472	1.301	67.81	1.831	98.16	1.991
22	4.69	1.342	71.84	1.856	98.14	1.991
24	4.898	1.38	76.23	1.882	98.11	1.991

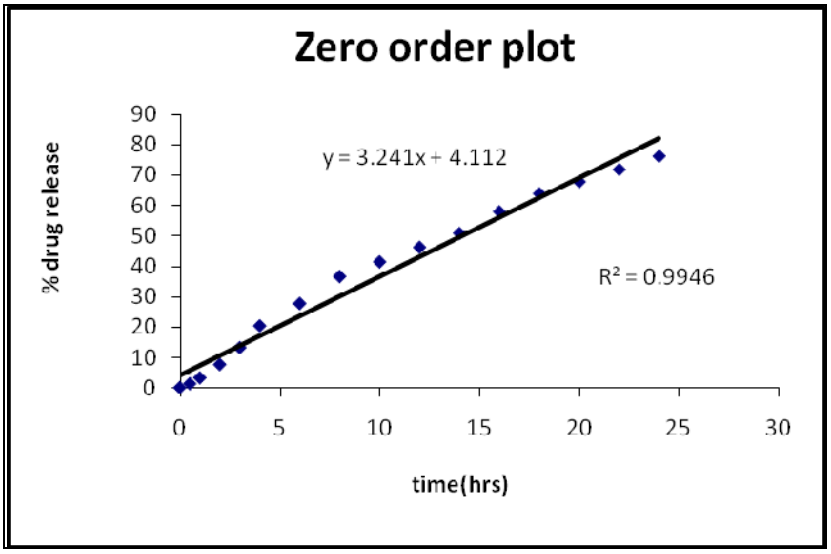


Fig 28 (a)

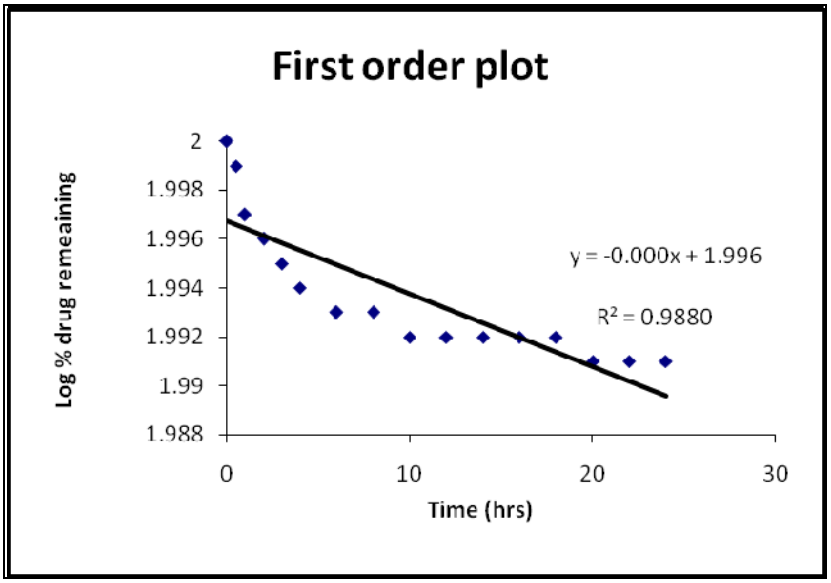


Fig 28 (b)

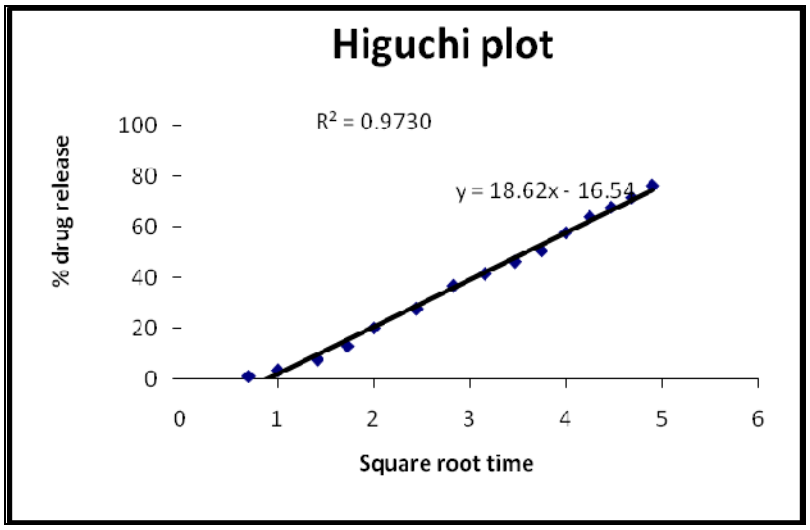


Fig 28 (c)

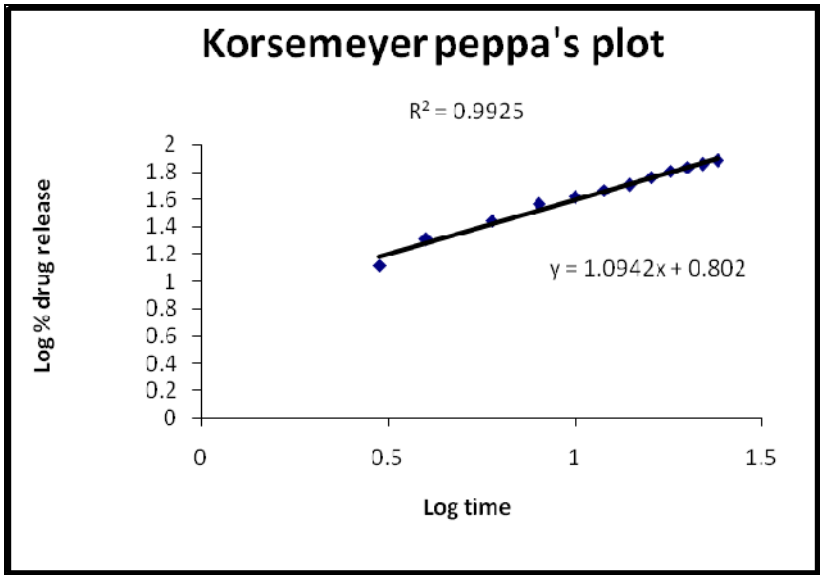


Fig 28 (d)

Table 30 Compartivie study for diffusion of nevirapine nanoparticles

Time (hrs)	Cumulative Percent Drug Release		
	F1	**F2**	**F3**
0	0	0	0
0.5	1.739	1.376	1.304
1	4.922	3.500	3.241
2	9.686	8.777	7.618
3	15.485	13.932	13.157
4	25.535	22.823	20.375
6	30.182.	29.536	27.729
8	37.394	36.997	36.604
10	43.129	42.859	41.435
12	49.996	48.437	46.240
14	52.238	51.110	50.790
16	58.491	57.810	57.925
18	68.825	67.383	63.864
20	77.572	69.360	67.817
22	84.789	75.762	71.840
24	92.407	83.432	76.239

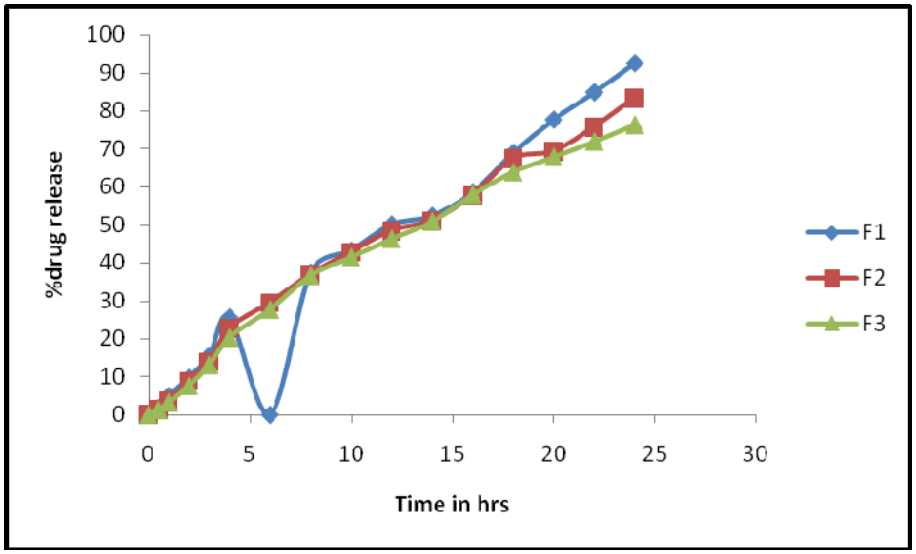


Fig 29 COMPARATIVE DIFFUSION PROFILE

Table 31 In vitro release kinetic data nevirapine loaded nanoparticles (DIFFUSION)

Formulation code	First order "R^2"value	Zero order "R^2"value	Higuchi plot "R^2"value	Peppas plot "R^2"value	n value
F1	0.9754	0.9865	0.9759	0.9813	1.0778
F2	0.9821	0.9890	0.9802	0.9886	1.0828
F3	0.9880	0.9946	0.9730	0.9925	1.0942

K_0 - Zero order rates constant

K_1 – First order rate constant

R – Coefficient of correlation

N – Diffusion exponent

The regression co-efficient values are zero order plots wew compared. It was observed that the "R" value of zero order plit were in the range of 0.9865 to 0.9946 from all the three formulations. The "R" values of first order plot were in the range of 0.9754 to 0.9880, based on the highest regression values (R), the best fit model for F1, F2 and F3. Followed zero order release. The "R" value of the linear regression for korsmeyer plot were found in the range of 0.9813 to 0.9925 for all the formulation indicating that the data fit into the korsmeyer plot model well, and the drug release was found to be predominantely controlled by swelling process.

When the slope n values of formulation F1,F2 and F3 in korsmeyer equation (F1=1.0778, F2=1.0828 and F3=1.0942) were compared, it was observed that increasing polymer concentration in the nanoparticle led to increase in the slope value from 0.7082 to 0.7952 indicating that the drug release by case II transport mechanism. (n>1) class II transport mechanism which involves swelling controlled release of the drug.

Turkey – Kramer multiple camparision test : t_{90}

The release data were subject to ANOVA with Turkey- Kramer comparision test F2 showed release pattern as compared F1 ($p<0.05$) .Similar release behavior was observed between F3 and F1 ($p<0.001$) and between F3 and F2 ($p<0.05$).The result suggest that the slow drug release characterstics appear to be influenced by polymer concentration. Table 32,33.

Table 32 ANALYSIS OF VARIANTS

Formulation code	F1	F2	F3
Mean	40.767	38.015	36.631
Standard deviation	30.235	27.680	26.749
sample size (N)	20	20	20
std error of mean	7.559	6.920	6.687
Lower 95% conf. limit	24.660	23.269	22.381
upper 95% conf. limit	56.575	52.761	50.883
Minimum	0	0	0
Median	40.255	39.920	39.015
Maximum	92.400	83.420	76.230
Normality test KS	0.1110	0.1205	0.1225
Normality test P value	>0.10	>0.10	>0.10
Passed normality test	yes	yes	yes

Table 33 Turkey – Kramer multiple comparison test: t_{90}

Comparision	Mean difference	"q" value	"p" value
F1 Vs F2	2.753	4.401	P< 0.05
F1 Vs F3	4.136	6.612	P< 0.001
F2 Vs F3	1.383	2.211	P> 0.001

The **"**P" value is <0.0001, considered extremely significant. Variation among column means is significantly greater than expected by chance.

If the value q is greater than 3.486 then the p value is less than 0.05.

HPLC graphs

Chromatograms of Nevirapine alone

0.5 hr

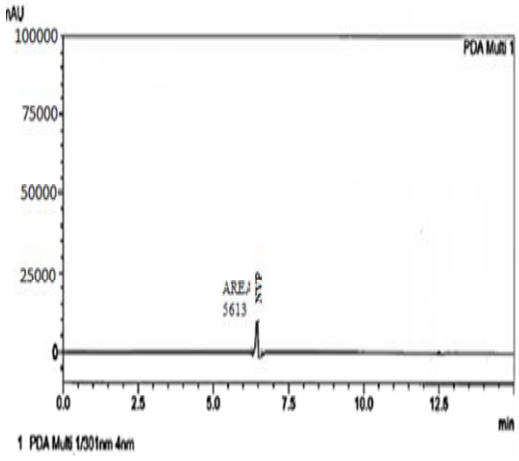


Fig 30 (a)

1hr

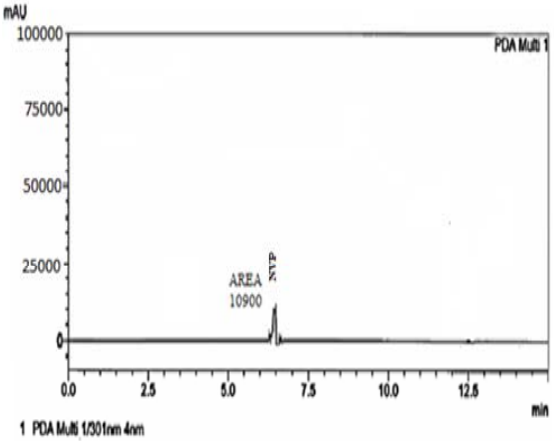


Fig30 (b)

1.5 hr

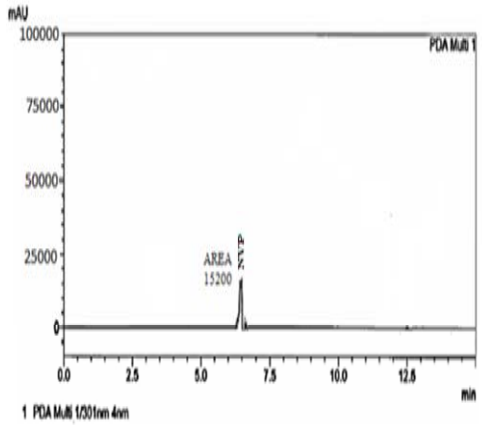


Fig 30 (c)

2 hr

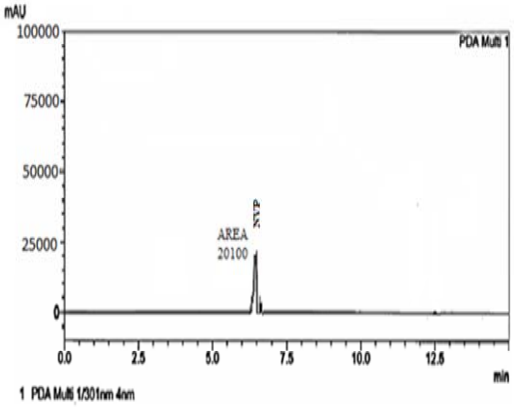


Fig 30 (d)

2.5 hr

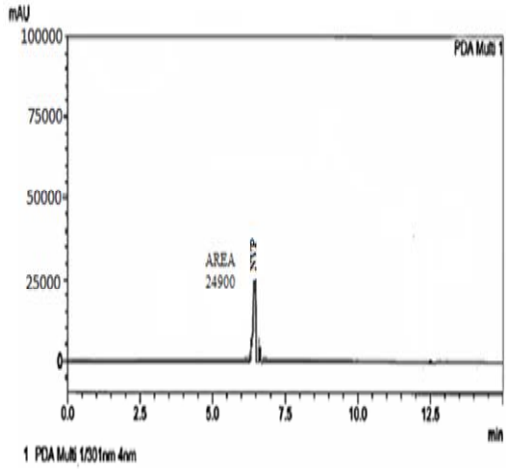


Fig 30 (e)

3 hr

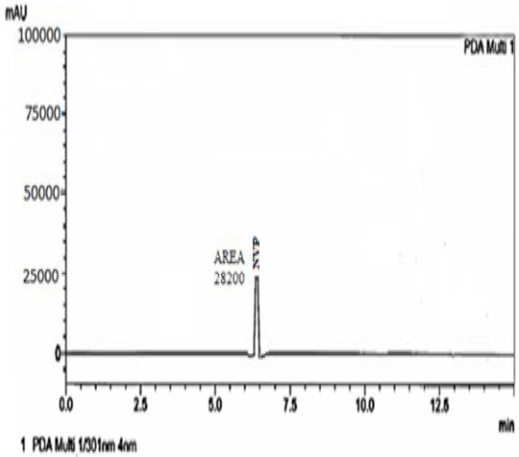


Fig 30 (f)

3.5 hr

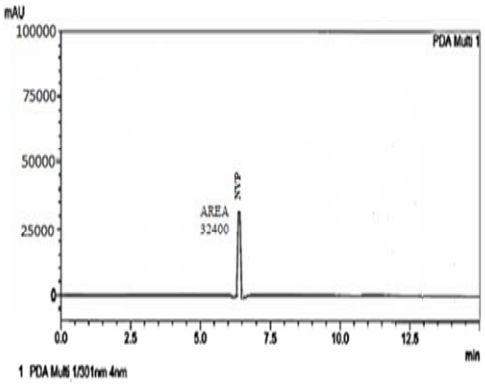


Fig 30 (g)

4 hr

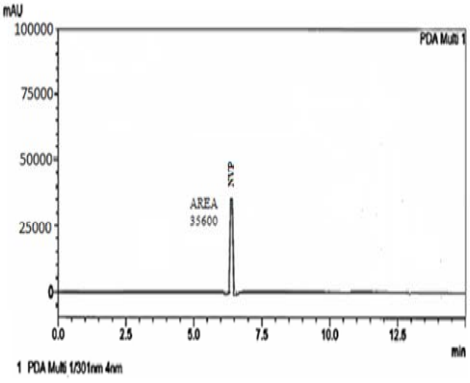


Fig 30 (h)

5 hr

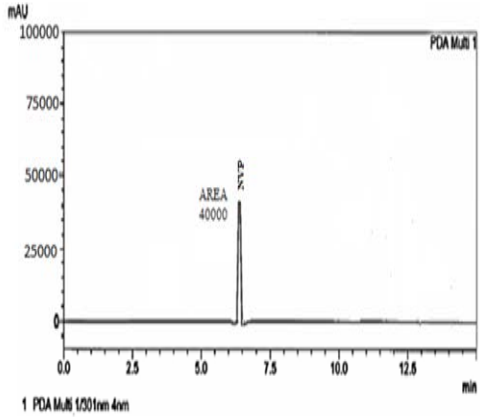


Fig 30 (i)

6 hr

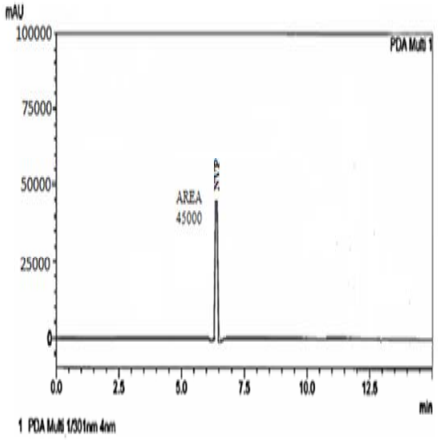


Fig 30 (j)

8 hr

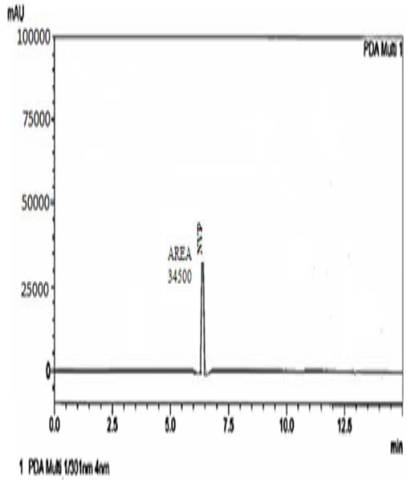


Fig 30 (k)

10 hr

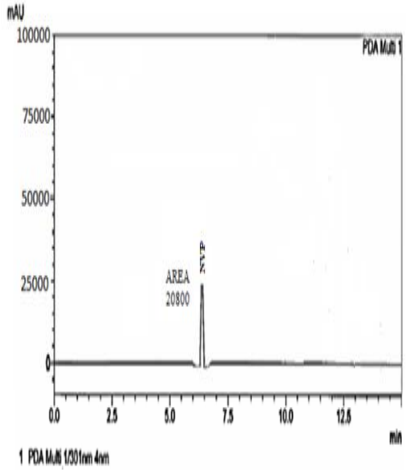


Fig 30 (l)

12.0 hr

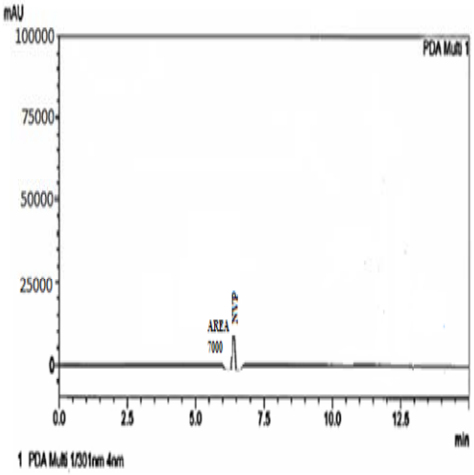


Fig 30 (m)

24.0 hr

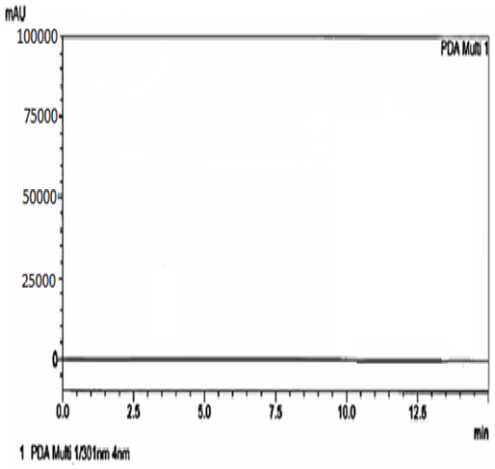


Fig 30 (n)

Chromatograms of Nevirapine Nanoparticles

0.5 hr

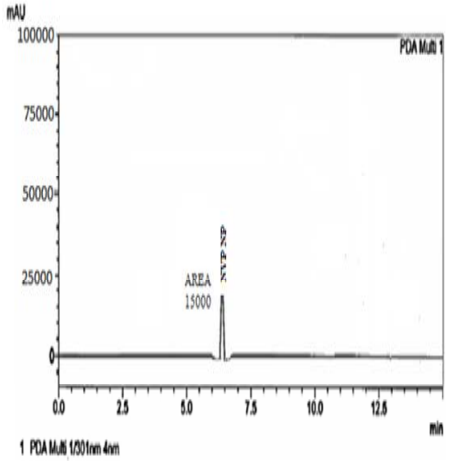


Fig 31 (a)

1 hr

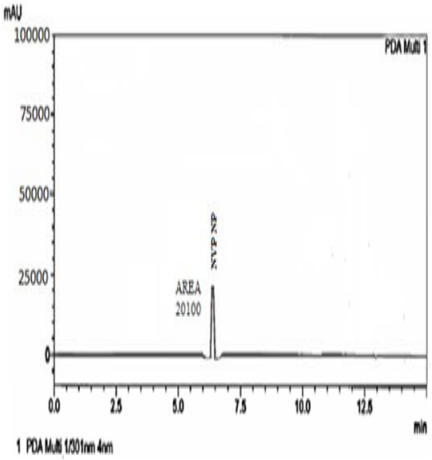


Fig 31 (b)

1.5 hr

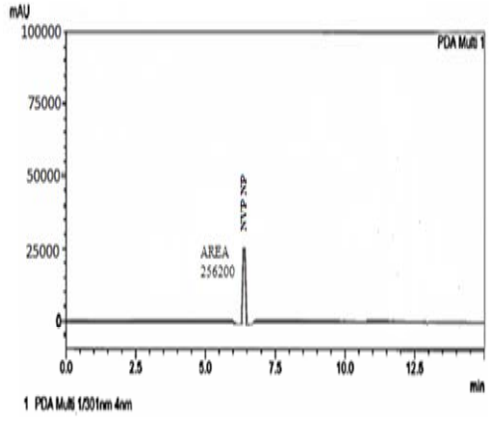


Fig 31 (c)

2 hr

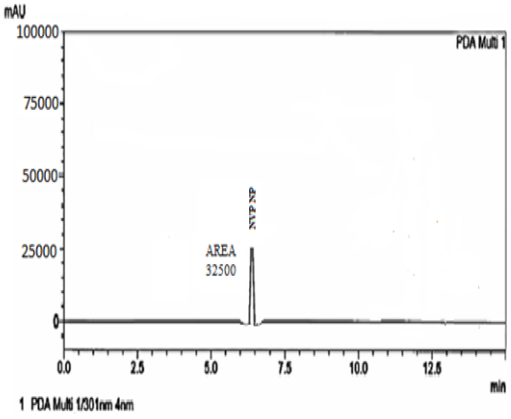


Fig 31 (d)

2.5 hr

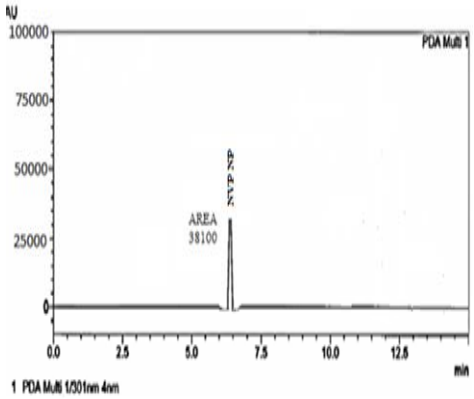


Fig 31 (e)

3 hr

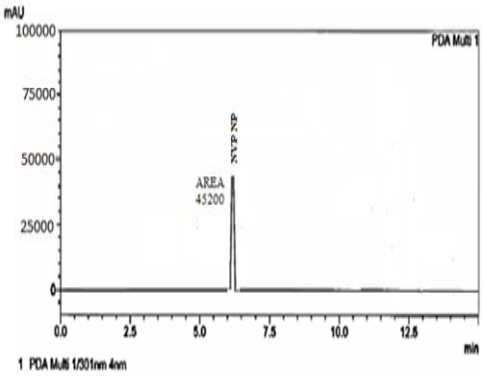


Fig 31 (f)

3.5 hr

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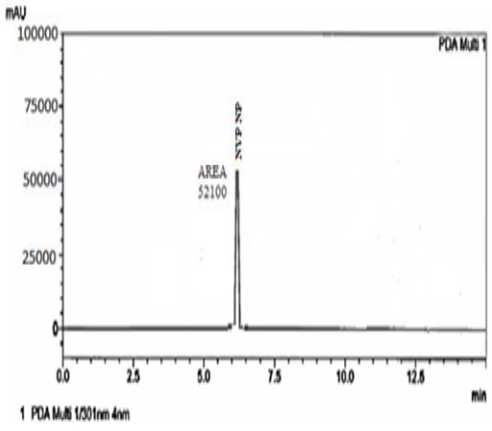

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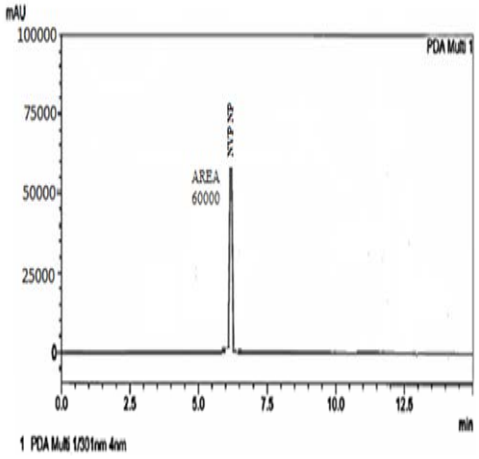

5HR

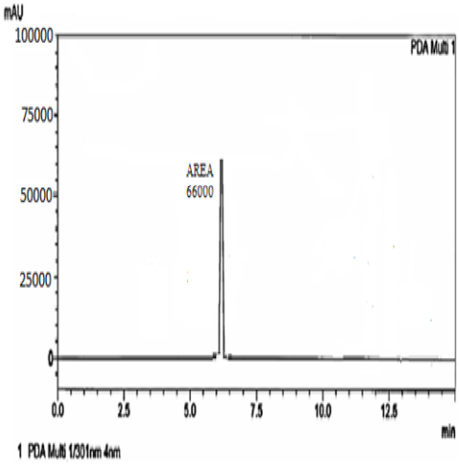

Fig 31 (i)

6HR

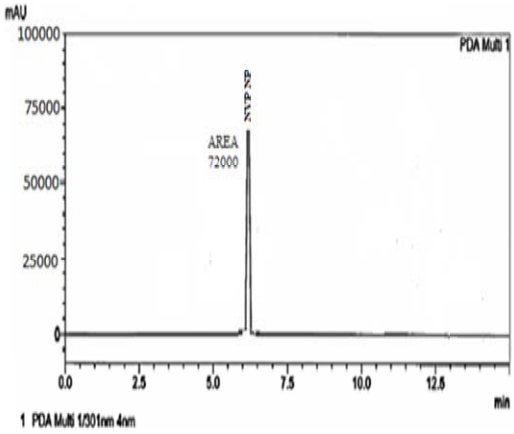

Fig 31 (j)

8HR

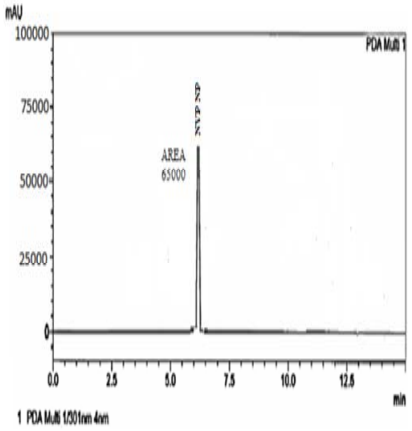

Fig 31 (k)

10HR

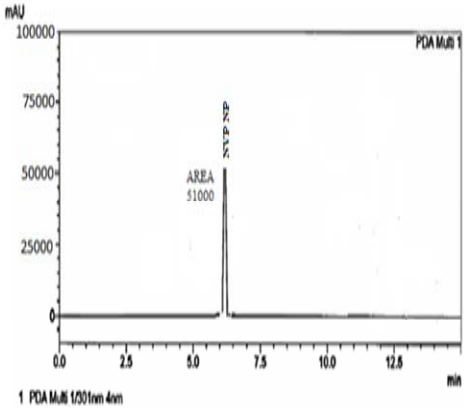

Fig 31 (l)

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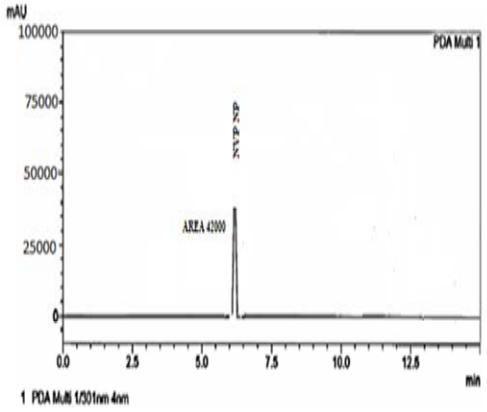

Fig 31 (m)

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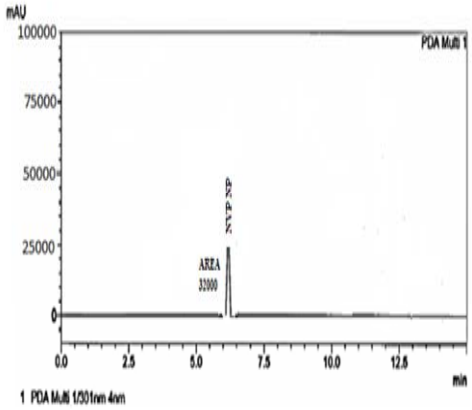

Fig 31 (n)

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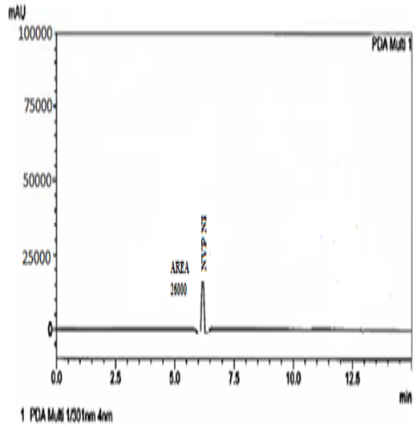

Fig 31 (o)

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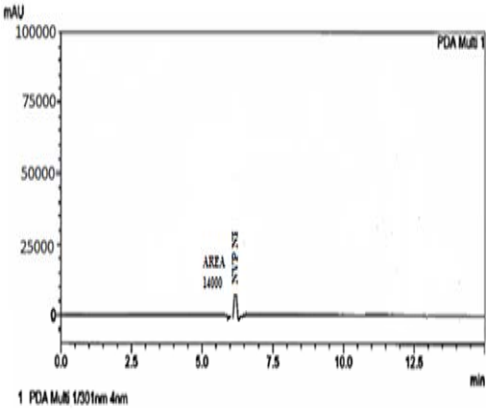

Fig 31 (p)

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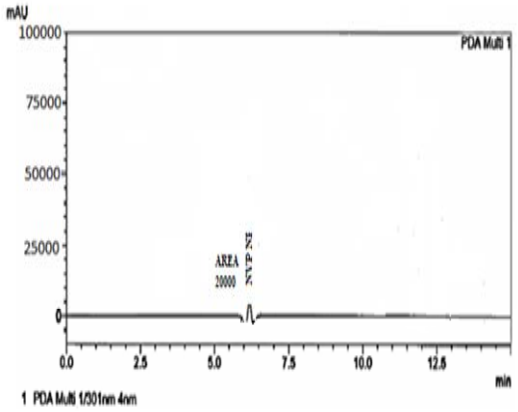
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PDA Multi 1
AREA
20000
0.0
2.5
5.0
7.5
10.0
12.5
min
1 PDA Multi 1/301nm 4nm

Fig 31 (q)

Table 34 Peak area of test plasma samples of Group I at various time intervals

Peak area of Nevirapine

Time (hr)	Peak area					
	1	2	3	4	5	6
0.5	5613	4314	6096	7291	8695	5790
1.0	10920	10520	11120	11500	10200	10620
1.5	15920	14800	16180	16720	16900	15900
2.0	20100	21500	21720	20900	20780	20600
2.5	24900	25100	21720	20900	20780	25180
3.0	28900	29720	27600	30100	28620	28700
3.5	32400	35000	33200	34900	32600	32750
4.0	35600	36200	36790	35490	34600	35910
5.0	40100	41500	42600	440600	43500	41200
6.0	45600	45720	45900	45600	44900	28950
8.0	34600	33191	30161	32191	33397	29500
10.0	20900	21200	21600	22800	21500	21800
12.0	10900	11200	12500	13600	15600	14900
24	0	0	0	0	0	0

Table 35 Peak area of Nevirapine nanoparticles

Time (hr)	Peak area					
	1	2	3	4	5	6
0.5	15200	16720	15910	17200	15920	18610
1.0	21900	20700	21200	25600	24600	25700
1.5	25620	27510	28900	29500	25720	26100
2.0	32600	33100	34600	35200	31700	32720
2.5	25620	27510	28900	29500	25720	26100
3.0	45200	45600	45720	46100	47200	45100
3.5	52100	53600	51900	54240	56920	55710
4.0	60720	60900	61290	62500	61900	64590
5.0	66720	67900	68920	69920	69790	68720
6.0	72500	73400	71600	73600	75200	71900
8.0	65000	66900	67500	68390	69310	65582
10.0	52100	53500	54600	55700	54720	55600
12.0	42800	43550	46800	48900	49500	47600
14.0	32500	34800	33500	37600	30900	35400
16.0	26500	24800	23700	28900	21400	25100
20.0	20800	20500	20900	21600	22400	20000
24.0	15100	14600	14000	14500	14800	16900

Table 36 Plasma concentration of test samples in Group I at various time intervals

Plasma concentration of Nevirapine

Time(hr)	Animals						Mean±S.E.M
	1	2	3	4	5	6	
0.5	0.05	0.15	0.06	0.14	0.05	0.15	0.1±0.02098
1.0	0.3	0.9	0.5	0.7	0.4	0.8	0.6±0.09661
1.5	1.0	2.0	1.4	1.6	1.3	1.7	1.5±0.14140
2.0	1.85	2.7	2.0	1.9	2.3	1.98	2.1±0.13220
2.5	3.0	3.4	2.9	3.5	3.1	3.3	3.2±0.09611
3.0	4.0	4.4	4.1	4.3	3.9	4.5	4.5±0.14240
3.5	5.0	4.9	4.8	5.4	5.3	5.2	5.1±0.09661
4.0	6.0	5.8	5.7	6.1	6.2	5.6	5.9±0.09661
5.0	6.8	6.4	6.6	6.9	6.2	6.0	6.5±0.14240
6.0	5.6	6.0	5.7	5.5	6.1	5.9	5.8±0.09661
8.0	4.0	4.2	3.9	4.3	3.8	4.4	4.1±0.09661
10.0	1.85	2.7	2.0	1.9	2.3	1.98	2.1±0.13220
12.0	0.05	0.15	0.06	0.14	0.05	0.15	0.1±0.02098
24.0	0	0	0	0	0	0	0

Table 37 Pharmacokinetic parameters of Nevirapine alone

S.no	Parameters	Animals						Mean±S.E.M
		1	2	3	4	5	6	
1	Cmax	6.8	6.4	6.6	6.9	6.2	6	6.85±0.15940
2	Tmax	4.5	4.5	4.5	4.5	4.0	4.5	4.89±0.08333
3	AUC_{0-24}	30.15	36.42	30.63	34.66	31.95	33.78	34.26±0.76640
4	k_{eli} (h^{-1})	0.344	0.335	0.322	0.358	0.339	0.345	0.304±0.004
5	$AUC_{0-\infty}$	45.44	54.51	46.27	51.90	46.07	50.13	49.05±1.51400

Table 38 Plasma concentration of Nevirapine Nanoparticles

Time(hr)	Animals						Mean±S.E.M
	1	2	3	4	5	6	
0.5	0.04	0.05	0.06	0.03	0.05	0.07	0.05±0.005774
1.0	0.3	0.2	0.4	0.4	0.3	0.2	0.3±0.036510
1.5	0.51	0.52	0.53	0.50	0.52	0.54	0.52±0.005774
2.0	1.04	1.05	1.06	1.03	1.05	1.07	1.05±0.05774
2.5	1.4	1.5	1.6	1.3	1.5	1.7	1.5±0.057740
3.0	2.0	2.1	2.2	1.9	2.1	2.3	2.1±0.057440
3.5	2.4	2.5	2.6	2.3	2.5	2.7	2.5±0.057740
4.0	3.0	3.1	3.2	2.9	3.1	3.3	3.1±0.057440
5.0	3.8	3.9	4.0	3.7	4.0	4.2	3.9±0.071490
6.0	4.1	4.2	4.3	4.0	4.2	4.4	4.2±0.057740
8.0	7.0	7..1	7.2	6.9	7.1	7.3	7.1±0.071490
10.0	6.4	6.5	6.6	6.3	6.5	6.7	6.5±0.057740
12.0	0.05	5.8	5.9	5.6	5.8	6.0	5.8±0.057740
14.0	4.0	4.1	4.2	3.9	4.1	4.3	4.1±0.057440
16.0	3.0	3.1	3.2	2.9	3.1	3.3	3.1±0.057440
20.0	1.4	1.5	1.6	1.3	1.5	1.7	1.5±0.057740
24.0	0	0.1	0.2	0	0.1	0.3	0.1±0.047730

Table 39 Pharmacokinetic parameters Nevirapine nanoparticles

S.no	Parameters	Animals						Mean±S.E.M
		1	2	3	4	5	6	
1	Cmax	7.0	7.1	7.2	6.9	7.1	7.3	7.1±0.058
2	Tmax	8.0	8.0	8.0	8.0	8.0	8.0	8.0±0.00
3	AUC_{0-24}	82.69	84.78	87.12	81.05	85.01	87.48	84.69±1.017
4	k_{eli} (h^{-1})	0.14	0.136	0.132	1.146	0.135	0.128	0.14±0.003
5	$AUC_{0-\infty}$	101.34	120.46	110.78	115.52	125.67	109.93	113.9±3.50

Table 40 Comparative Pharmacokinetic parameters of Nevirapine and Nevirapine Nanoparticles

S.no	**Parameters**	**Nevirapine**	**Nevirapine Nanoparticles**
1	Cmax	6.85±0.15940	7.1±0.058
2	Tmax	4.89±0.08333	8.0±0.00
3	AUC_{0-24}	34.26±0.76640	84.69±1.017
4	k_{eli} (h^{-1})	0.304±0.004	0.14±0.003
5	$AUC_{0-\infty}$	49.05±1.51400	113.9±3.50

9.5 Pharmacikinetics of nevirapine and nevirapine nanoparticles:

9.5.1 C_{max} of nevirapine alone and nevirapine nanoparticles

The mean C_{max} values of NVP amd nevirapine nanoparticles are respectively 6.85±0.15940 and 7.1±0.058 . There is extremely significant (P<0.001) increase in C_{max} of NVP alone when compared with nevirapine nanoparticles group and extremely significant (P<0.001) increase in C_{max} when compared with NVP group.

Table. 41 C_{max} of nevirapine and nevirapine nanoparticles

S.NO	GROUP	C_{max}(µg/ml) Mean±S.E.M
1	NVP alone	6.85±0.15490[b]
2	NVP NP	7.1±0.058[a]

The values are expressed as mean±SEM; a=P<0.001; b=P<0.001 when compared with NVP alone, NVP NP group (one way ANOVA followed by Tukey's multiple comparision test)

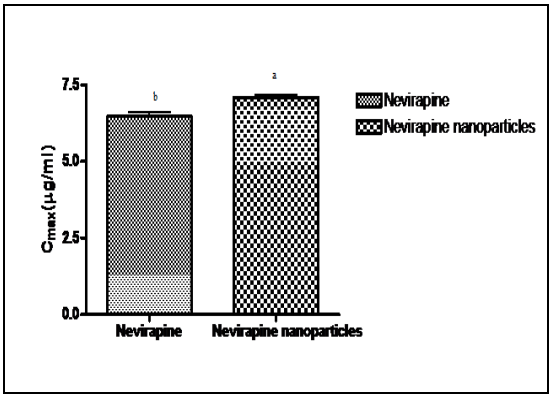

Fig 32 C_{max} of in NVP and nevirapine nanoparticles

C_{max} of NVP alone, NVP NP.The values are expressed as mean±SEM; ***P<0.001; *** P<0.001 when compared with NVP alone and NVP NP group, (one way ANOVA followed by Tukey's multiple comparision test).

9.5.2 T_{max} of Nevirapine (NVP) and Nevirapine nanoparticles (NVP NP)

The mean T_{max} values of NVP, NVP NP are respectively 4.89±0.08333 and 8.0±0.00. There is extremely significant (P<0.001) increase in C_{max} of NVP alone when compared with nevirapine nanoparticles group and extremely significant (P<0.001) increase in C_{max} when compared with NVP group.

Table. 42 T_{max} of NVP ,and NVP NP

S.NO	GROUP	$C_{max}(hr^{-1})$ Mean±S.E.M
1	NVP alone	$4.89±0.0833^{b}$
2	NVP NP	$8.0±0.00^{a}$

The values are expressed as mean±SEM; a=P<0.001; b= P<0.001 when compared with NVP alone, NVP NP group (one way ANOVA followed by Tukey's multiple comparision test).

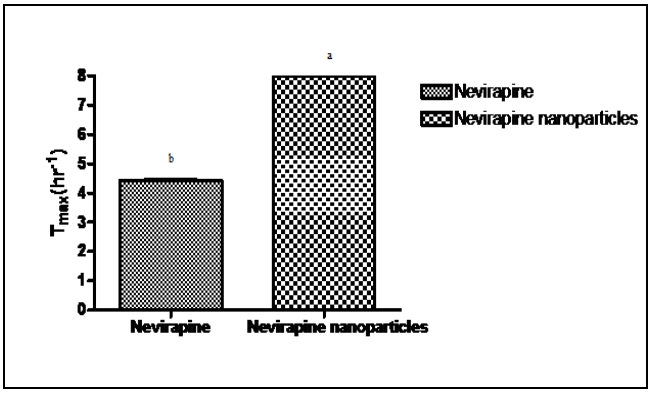

Figure 33 T_{max} of NVP and NVP NP

T_{max} of NVP alone, NVP NP.The values are expressed as mean±SEM; a=P<0.001;b= P<0.001 when compared with NVP alone and NVP NP group, (one way ANOVA followed by Tukey's multiple comparision test).

9.5.3 AUC_{0-24} of NVP and NVP NP

The mean AUC_{0-24} values of NVP and NVP NP are respectively 34.26±0.76640 and 84.69±1.017. There is extremely significant (P<0.001) increase in C_{max} of NVP alone when compared with NVP NP group and extremely significant (P<0.001) increase in AUC_{0-24} of NVP NP group when compared with NVP group.

Table 43 AUC_{0-24} of nevirapine alone and nevirapine nanoparticles

S.NO	GROUP	AUC_{0-24} Mean±S.E.M
1	NVP alone	34.26±0.76640[b]
2	NVP NP	84.69±1.017[a]

The values are expressed as mean±SEM; a=P<0.001; b= P<0.001 when compared with NVP alone, NVP NP group. (one way ANOVA followed by Tukey's multiple comparision test)

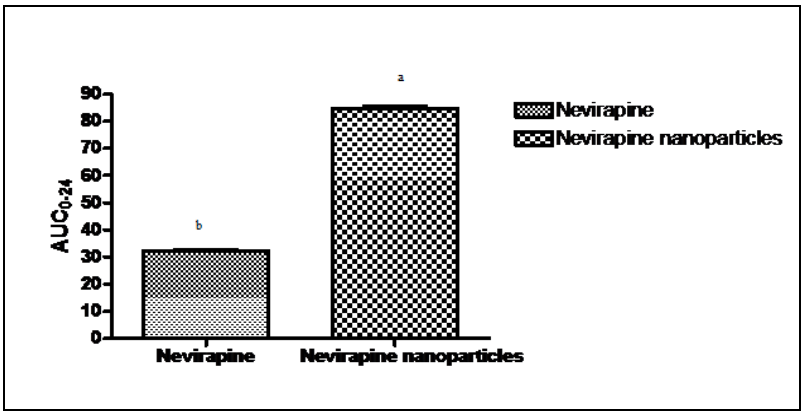

Figure 34 AUC_{0-24} of nevirapine alone and nevirapine nanoparticles

AUC_{0-24} of nevirapine in NVP alone nevirapine nanoparticles; The values are expressed as mean±SEM; a=P<0.001; b= P<0.001 when compared with NVP alone, NVP NP group. (one way ANOVA followed by Tukey's multiple comparision test).

9.5.4 K_e (hr^{-1}) of nevirapine alone, and nevirapine nanoparticles

The mean K_e (hr^{-1}) values of NVP, NVP NP are respectively 0.304±0.004 and 0.14±0.003 . There is very significant (P<0.001) increase in C_{max} of NVP alone when compared with NVP NP group .

Table. 44 K_e (hr^{-1}) of in NVP alone, NVP NP

S.NO	GROUP	K_e (hr^{-1}) Mean±S.E.M
1	NVP alone	0.304±0.004 [b]
2	NVP NP	0.14±0.003 [a]

The values are expressed as mean±SEM; a=P<0.01; b= P<0.001 when compared with NVP alone, NVP NP group. (one way ANOVA followed by Tukey's multiple comparision test)

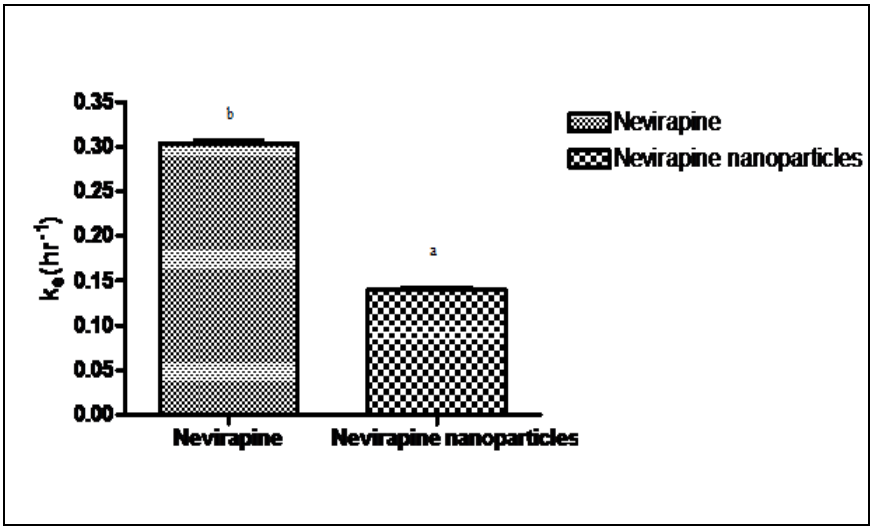

Figure 35 K_e (hr^{-1}) of in NVP alone, NVP NP

K_e (hr^{-1}) of nevirapine alone, NVP NP. The values are expressed as mean±SEM; a=P<0.01; **b= P<0.001 when compared with NVP alone, NVP NP group. (one way ANOVA followed by Tukey's multiple comparision test).

9.5.5 $AUC_{0-\infty}$ of nevirapine and nevirapine nanoparticles

The mean $AUC_{0-\infty}$ values of NVP and NVP NP are respectively 49.05±1.51400 and 113.9±3.50 . There is extremely significant (P<0.001) increase in C_{max} of NVP alone when compared with NVP NP group.

Table 45 $AUC_{0-\infty}$of nevirapine and nevirapine nanoparticles

S.NO	GROUP	$AUC_{0-\infty}$ Mean±S.E.M
1	NVP alone	49.05±1.51400 [b]
2	NVP NP	113.9±3.50 [a]

The values are expressed as mean±SEM; a=P<0.001; b= P<0.001 when compared with NVP alone, NVP NP group. (one way ANOVA followed by Tukey's multiple comparision test).

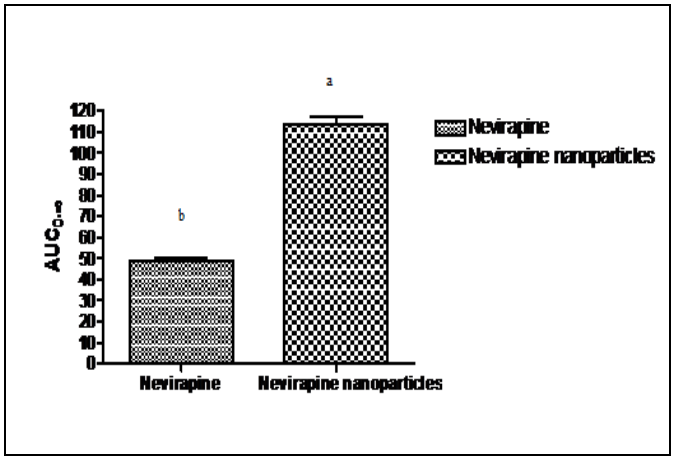

Figure 36 $AUC_{0-\infty}$of nevirapine and nevirapine nanoparticles

$AUC_{0-\infty}$of nevirapine and nevirapine nanoparticles. The values are expressed as mean±SEM; a=P<0.001; b= P<0.001 when compared with NVP alone, NVP NP group. (one way ANOVA followed by Tukey's multiple comparision test).

10.0 DISCUSSION

The results of the present study demonstrated that delivery of nevirapine as nanoparticles loaded in humanserumalbumin (HSA) could be beneficial to improve the physical characteristics of the drug and thus improve the bio availability of nevirapine. Nevirapine is recommended in combination therapy with other anti HIV drugs in the initial regimen to reduce impacts on the clinical implications of drug resistance **(Mugavero C.B Hicks et al 2004)[72].**

Currently nevirapine is available as tablets and pediatric oral suspensions. Owing to poor bioavailability nevirapine may not control the HIV infection effectively. Focusing on this in the present study an attempt was made to develop nanoparticles of nevirapine using HSA as a polymer. Nanoparticles were prepared by desolvation method in which no organic solvent was employed and as such the developed nanoparticles are free of any toxic effects that are common with organic solvents used in the preparation method. In addition, the desolvation- chemical crosslinking method can keep drug activity while enveloping the drug in the nanoparticles. **(D.Chen et al 2010)[73]**

The encapsulation efficiency and percentage yield of the nanoparticles were found influenced by HSA concentration; both parameters were increased with increasing HSA concentration. During the formation of nanoparticles the NVP might be undergoing shift from crystallanity to amorphous in the polymer HSA and therefore the increasing encapsulation efficiency and percentage yield with increase in HSA concentration. The DSC thermogram showed characteristic change in the endothermic peak of nevirapine showing amorphous form of the drug that helps in influencing the encapsulation efficiency and percentage yield of nanoparticles. The particle was found discrete and irregular at all concentration of polymer, however with increase in size of particles as the concentration of HSA increased. The particle size increased with increase in HSA concentration. The particle site of nanoparticles depend upon that techniques followed for preparation of nanopartilces and other factors such as the type of crosslinking agent and the desolvating agent used. It has been reported that the amount of desovating agent ethanol in the desolvation process was found to control particle size **(Langer K et al 2003)[74].** In this study the amount of desolvating agent ethanol was used at a fixed volume and therefore its influence on the size of the nanoparticles is unlikely. It has also been reported that rate of ethanol addition also influence particle size; however in the study ethanol addition was not measured and therefore its influence on the site of nanoparticles could not be established clearly. The size of the

nanoparticles ranged from 298 ± 025nm to 698 ± 0.52 nm (Table 13). An increase in particle site was evident with increase in HSA concentration. As other variables such as the amount of desolvating agent ethanol, the rate of addition of ethanol, the stirring speed used were not controlled in the preparation of nanoparticles in the present study and therefore the role of HSA concentration (though influenced particle size) on the size of nanoparticles could not be clearly defined.

The PDI of nanoparticles ranged between 0.195 and 0.435 (Table 13) which indicates a homogenous dispersion of the drug; the PDI was found increased with increasing the HSA concentration indicating that higher concentration of the polymer detrimental to homogenous dispersion of the drug. This may be another reason pointing to suggest that the particle size of the nanoparticles depending on the HSA concentration.

Surface characterstics of the nanoparticles greatly influence their intereaction with the biological memberane, besides the stability of nanoparticles. Zeta potential is an index of the stability of the nanoparticles under most conditions, the higher the absolute value of the zeta potential of the nanoparticles, the larger the charge on their surface, leading to stronger repulsive interaction between the dispersed nanoparticles and higher stability and more uniform size. It has also been demonstrated that a high potential value of above ±25mv, ensures a high energy barrier that stabilizes the nanosuspension **(Muller R.H. et al)**[75]**.** A lower zeta potential (less than 0.25mv) observed in the formulations F_2 & F_3 may be another factor influencing the particle site of nanoparticles in these formulations. The nano size initially formed during the preparation of nanoparticles formed aggregates to grow in size due to instability of the nanoparticles with low zeta potential which was evident in F_2 & F_3.

A variable dissolution profile of formulations F_1, F_2 and F_3 was observed. All formulations showed slow dissolution of nevirapine. The mean dissolution time of F_1, F_2 & F_3 was 27.69, 27.63 and 28.28 respectively and each value was found to be close to that of other, and as such the mean dissolution time of nevirapine was comparatle between F_1, F_2 & F_3. However the percentage of nevirapine release was highest 91.36% (Table 14 and fig 15) from F_1, and lowest 71.55 (Table 18, and fig 20) from F_3 formulation. The percentage of nevirapine release from F2 was 83.51 (Table 16 and fig 17) . All the formulations showed zero order release kinetic with r^2 value about 0.99. The variation in percent drug release at different time point intervals, in different formulations may be due to the HSA concentration used.The factors influencing the dissolution of nevirapine can be stated as follows. 1. Particle

size place as important role in influencing the dissolution of the drug in the environment. The particle size was least in F_1 298 nm(fig 14) as compared to that in F_2 495nm(fig 15) and F_3 698nm (fig 16). Therefore it is obvious that a higher percent of nevirapine was released from F_1 as compared to F_2 & F_3. Other factors such as pH of the environment can also influence the dissolution of drug. However in the present study all formulations showed slow dissolution which was found to be independent of pH of the environment. These observations suggest that HSA nanoparticles release the drug at a rate independent of the pH of the environment.

The in-vitro dissolution profile of F_1, F_2 & F_3 was also reflected in the diffusion study. The percentage drug diffused from F1, F2 & F3 was 92.40%. 83.43% and 76.23% (Table 24,26 and 28) respectively. These findings propose that factors particularly particle size of nanoparticles did also influenced the diffusion of drug which was found to be dependent upon the HSA concentration. There was no burst release of drug from all the formulations and showed a near perfect zero order release of the nanoparticles.

Based on the dissolution and diffusion characteristics the formulation F_1 was found to be the ideal formulation for further in-vivo study. According to FDA guidelines a controlled release formulation should release 0-20% at 4 h, 15-70% at 12 h and > 85% at 24h. Accordingly a formulation F_1 was found to satisfy the above requirement compared to F_2 & F_3 and as such the formulation F_1 was selected for the in-vivo pharmacokinetic study. The Cmax of F_1 was found to be greater than nevirapine alone ($P>0.001$). Similarly the AUCo-24 and AUCo-∞ of F_1 were significantly higher than that of neverapine alone and the difference was statically Significant ($P<0.001$).The above findings clearly indicates that the bioavailability of nevirapine was found to be better from nanoparticles as compared to nevirapine alone. The increase in bioavailabity of nevirapine nanoparticles can be attributed to the following factors. 1. The size of nanoparticles (F_1) is for lesser than the size of pure nevirapine which is normally in micron range. 2. The human serum albumin may help better intereaction of the loaded nevirapine with the biological membrane that facilities improved permeability of the drug. 3. The drug particle was in the amorphous state in the nanoparticles as observed from the results the DSC study since amorphous drug is more soluble than the pure crystalline nevirapine.

The Tmax of nanoparticles of (F_1) was significantly higher than that of nevirapine alone ($P<0.001$). This is because the concentration of the drug for absorption was slowed by

the effect of HSA. The Tmax of F_1 was increased, and this was further evident from the reduced Ke value of nanoparticles as compared that of nevirapine alone.

11.0 Conclusion

The present study reveals that nevirapine loaded human serum albumin(HSA) nanoparticles released the drug at slow and controlled rate with improved pharmacokinetics of nevirapine. The release rate and surface characterstics of nanoparticles was found to be influenced by the HSA concentration. Thus it can be concluded that nevirapine loaded- HSA nanoparticles is beneficial in improving bioavailability of drug as compared to conventional tablet dosage form. Further studies in humans are recommended for clinical outcome with this nanoparticle.

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